The American Journal of Medicine

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CARL R. HONIG, STEPHEN MARSH TENNEY AND PAUL V. GABEL	910
Using a variety of technics appropriate to measurement of the rapid circulatory changes which follow the administration of nitroglycerine, the authors reexamine the controversial aspects of the effects of this drug on coronary blood flow, cardiac output, ventricular work and myocardial efficiency. The results, in general, support the classic view that nitroglycerine relieves myocardial ischemia by increasing the delivery of oxygen rather than by reducing cardiac work and oxygen requirement. Action and reaction follow upon one another so closely, however, and in such complex hemodynamic interaction, that the data do indeed furnish an extraordinary illustration of the diversity of integrated responses during the unsteady state.	
Idiopathic Hypertrophic Subaortic Stenosis. Clinical, Hemodynamic and Angio- graphic Manifestations	
EUGENE BRAUNWALD, ANDREW G. MORROW, WILLIAM P. CORNELL,	

Maurice M. Aygen and Theodore F. Hilbish

It is becoming increasingly apparent that obstruction to left ventricular ejection may be caused not only by aortic valvular stenosis, supravalvular stenosis and subvalvular stenosis due to fibrous bands but also by diffuse or localized subvalvular muscle hypertrophy of the left ventricle. Eleven such cases are described. In patients with clinical, electrocardiographic and roentgenographic manifestations of obstruction of the left ventricular outflow tract, the abnormality in question is suggested by substantial systolic pressure gradients within the left ventricle, as determined by left heart catheterization, and confirmed by left ventricular angiocardiograms. The authors indicate that three subtypes of the disorder may exist, some familial. Recognition is important when operative correction of the obstructing lesion is under consideration. Surgery for idiopathic hypertrophic subaortic stenosis involves formidable problems but is shown to be feasible.

The Effects of Intermittent Positive Pressure Breathing on the Intrapulmonary Distribution of Inspired Air

> 946 GLORIA TORRES, HAROLD A. LYONS AND PETER EMERSON

Widespread use of intermittent positive pressure breathing (IPPB) in pulmonary and neuromuscular diseases has led to investigation of the mode by which such therapy is effective. The authors offer elaborate proof that the beneficial effects of IPPB on the intrapulmonary mixing of

Contents continued on page 5



Raudixin—the cornerstone of antihypertensive therapy—helps relieve the pressures in your patients—helps relieve the pressures on your patients / 50 and 100 mg. tablets whole root rauwolfia for exceptional patient response



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NUMBER SIX

gases are due to the increase in tidal volume obtained, and that IPPB is particularly valuable in patients unable to maintain or increase tidal volume (e.g., patients with emphysema). With flow rates commonly employed, no significant unevenness of ventilation was obtained.

Pulmonary Function in the Hamman-Rich Syndrome. The Abnormalities of Ventilation, Blood Gases and Diffusion at Rest and on Exercise

ROBERT A. B. HOLLAND AND RALPH B. BLACKET

Careful pulmonary function studies in five cases of Hamman-Rich syndrome confirmed previously recorded reduction in lung volumes and the frequent occurrence of hyperventilation on exercise and even at rest. When the arterial oxygen saturation was reduced, this could not be attributed solely to reduction in diffusing capacity; increased venous admixture also was present. Thus in this syndrome not only impaired diffusion but also increased physiological dead space and increased venous admixture all contribute to the deficits in pulmonary function.

Antibiotics and Terminal Pneumonia. A Postmortem Microbiological Study Yale Kneeland, Jr. and Katherine Mills Price 967

The authors bring together a wide range of interesting data on the nature of the organisms which, in this age of liberal antibiotic therapy, are associated with "terminal pneumonia." The pneumococcus, Group A hemolytic streptococcus and H. influenzae have virtually disappeared from the scene, replaced by the staphylococcus, and a variety of gram-negative bacilli, notably pyocyaneous. The implications of this shift in bacterial population are discussed, with special reference to the place of antibiotics in the armamentarium of "the ardent therapeutist who wishes to fight the longest possible rear-guard action in a hopeless cause."

Pulmonary Disease in Adults Associated with Unclassified Mycobacteria

Lynn C. Christianson and Hal J. Dewlett 980

Clinical, epidemiologic, therapeutic and pathologic data in twenty-five patients with pulmonary lesions from which the only known pathogenic organism isolated was an unclassified mycobacterium support the view that infection of the lungs by these mycobacteria constitutes a distinct clinical entity. While similar in many respects to tuberculosis, the clinical and pathologic picture may differ in the degree of infectiousness and the response to treatment. The source of the infection is not yet apparent and the therapy is not as satisfactory, with the drugs available, as in tuberculosis.

An Epidemic of Inhalation Anthrax, the First in the Twentieth Century. I. Clinical Features

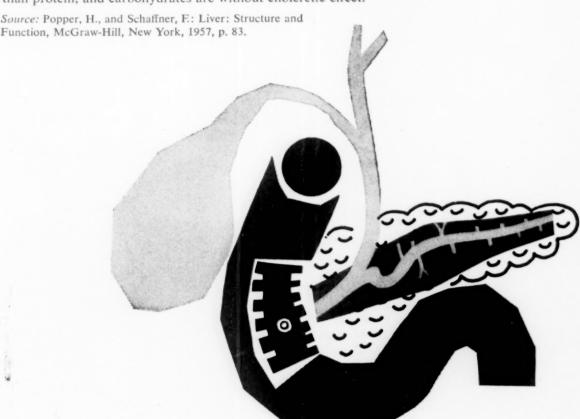
STANLEY A. PLOTKIN, PHILIP S. BRACHMAN, MILTON UTELL, FOREST H. BUMFORD AND MARY M. ATCHISON 992

An outbreak in a goat hair processing mill yielded five cases of inhalation anthrax (four fatal) and four cases of cutaneous anthrax. The results of intensive study of this epidemic are here recorded, with an interesting account of the fulminating course of inhalational infection terminating in hemorrhagic mediastinitis, pneumonitis and leptomeningitis. The problems of diagnosis and antibiotic therapy are informatively discussed.

Contents continued on page 7

how does diet affect the production of bile?

High-protein diets produce the greatest bile flow. Fat is a weaker choleretic than protein, and carbohydrates are without choleretic effect.



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New Observations on the Sympathetic Postganglionic Mechanism.

J. H. BURN AND M. J. RAND 1002

This interesting study clarifies the physiologic role of norepinephrine, which has for some time been known to be stored chiefly in the terminations of postganglionic sympathetic fibers, and the nature of the peripheral effects of reserpine on the cardiovascular system. Based on this information, explanations are offered for certain clinical phenomena which have long been puzzling, such as the need sometimes for rapid increases in the dosage of norepinephrine given intravenously to combat peripheral vascular collapse. Interesting suggestions are made also in connection with the action of nicotine on the peripheral blood vessels.

Nitrogen Mustard and the Steroid Hormones in the Treatment of Inoperable Bronchogenic Carcinoma

JULIUS WOLF, PAUL SPEAR, RAYMOND YESNER AND MARY ELLEN PATNO 1008

A cooperative study of some 500 cases of inoperable bronchogenic carcinoma established that 63 per cent of patients treated with nitrogen mustard survived ninety days as compared with 51 per cent of patients given a placebo, a dubious gain, and even this possible advantage not sustained for longer periods. Cortisone seemed, if anything, to shorten the survival period. Testosterone, diethyl-stilbestrol and progesterone had no discernible beneficial effect.

A Study of the Inverse Relationship Between pKa and Rate of Renal Excretion of Phenylbutazone Analogs in Man and Dog

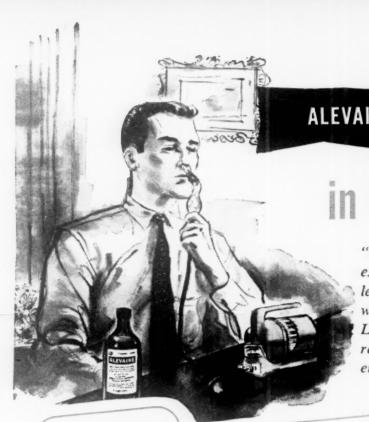
A. B. Gutman, P. G. Dayton, T. F. Yü, L. Berger, W. Chen, L. E. Sicam and J. J. Burns 1017

In a series of phenylbutazone analogs it was found that the more acidic compounds (lower pKa) were rapidly excreted whereas the less acidic compounds (higher pKa) were very slowly excreted by the kidney. Further investigation revealed that these differences were attributable to (1) a direct relationship between pKa and non-ionic back diffusion (which was more pronounced with high pKa compounds), and (2) an apparent inverse relationship between pKa and the rate of active tubular secretion of the compounds. Both combined to give the inverse relationship between pKa and rate of urinary excretion of drug which had been observed empirically. While the pKa is only one of several important factors involved, the relationships described may prove helpful in predicting the plasma half-life and excretory rate of acidic drugs.

Review

Nucleic Acids and Cancer David A. Goldthwait 1034

Dr. Goldthwait has brought together, in readily digestible form, a great deal of information concerning the nucleic acids, with special reference to their relationship to cancer, a most intriguing subject. He begins with a description of DNA, associated with the transmission of genetic information, and RNA, associated with protein biosynthesis. He then proceeds to a consideration of the general mechanisms of cell differentiation, and cell division in normal and neoplastic tissues, pointing up the loss of control of the machinery of cell division in cancer cells. The nature and mechanisms of action of specific carcinogens, which presumably affect the DNA genetic material, are next discussed. Finally, there is a critical account of available information concerning the nature and sites of metabolic block produced by a variety of agents employed in cancer chemotherapy.



ALEVAIRE® aerosol in the home

in bronchiectasis-

"Thick, yellow, solid sputum which had been expectorated with difficulty became thin, colorless and liquid sputum which was expectorated with ease and gradually diminished in volume. Labored breathing and insomnia, . . . soon were replaced by easy respiration and ability to enjoy normal restful sleep."*

CASE REPORT

A typical Alevaire case history-C. S., 31 year old male with bronchiectasis and sinusitis, had had pneumonia six times. He had a continuous thick purulent postnasal drip and thick, yellowish green sputum; he expectorated at least a cupful of sputum each morning on arising. The patient was weak and debilitated, with chills and low grade fever. Bronchograms revealed advanced bronchiectasis. Antibiotics, postural drainage and expectorant cough mixtures had not helped.

Alevaire therapy was begun with one hour of direct nasal inhalation every day. After the first treatment the patient expectorated a large amount of sputum and definitely breathed easier. The nasal passages began to open, and with subsequent treatments both the sinusitis and the bronchiectasis improved. He began to breathe easier through the nose and to expel bronchial secretions more readily. His appetite improved and he felt stronger.

At the end of fourteen days he was almost completely symptom free. Alevaire was continued each night for short periods at bedtime, and the patient remained completely free of symptoms except for a light morning *Miller, J.B., et al.: Ann. Allergy, 12:611, Sept.-Oct., 1954. expectoration.

Alevaire is supplied in bottles of 60 cc. for intermittent therapy and in bottles of 500 cc. for continuous inhalation therapy.

has been dramatically effective in:

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- pertussis pneumonia bronchial asthma
- emphysema bronchiectasis lung abscess
- pneumoconiosis
 smoke, kerosene poisoning
- poliomyelitis (respiratory complications)
- routine oxygen therapy tracheotomy
- prevention of postoperative pulmonary complications

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Clinico	patholo	gic C	onference

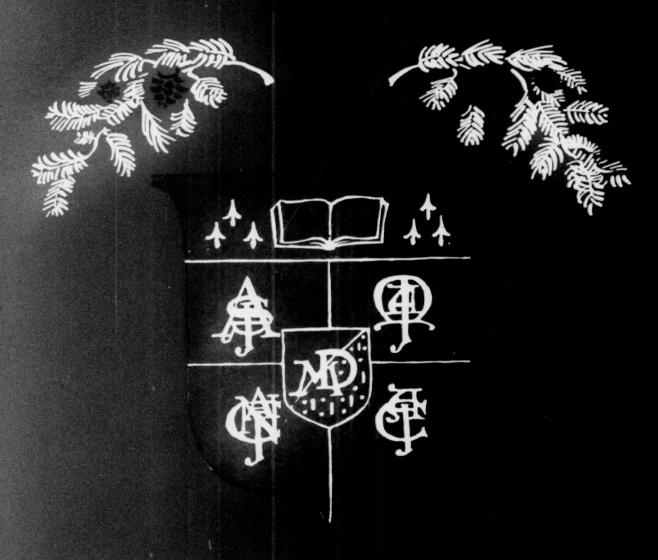
Arthralgias, Thrombophlebitis, Weight Loss, Pleural Effusion and Elevated Alkaline 1060 Phosphatase

Clinicopathologic Conference (Washington University School of Medicine).

Case Report

Stromal Endometriosis	Involvi	ing the	Hear	t										
		FELSON			McG	UIRE	AND	Рни	LIP	W	ASSE	RMA	IN	1072
An instructive case.														
Author Index														1077
Subject Index														1079

Advertisers' Index on Pages 129 and 130



Season's Greetings

a new, improved, more potent relaxant for anxiety and tension

· effective in half the dosage required with meprobamate

 much less drowsiness than with meprobamate, phenothiazines, or the psychosedatives

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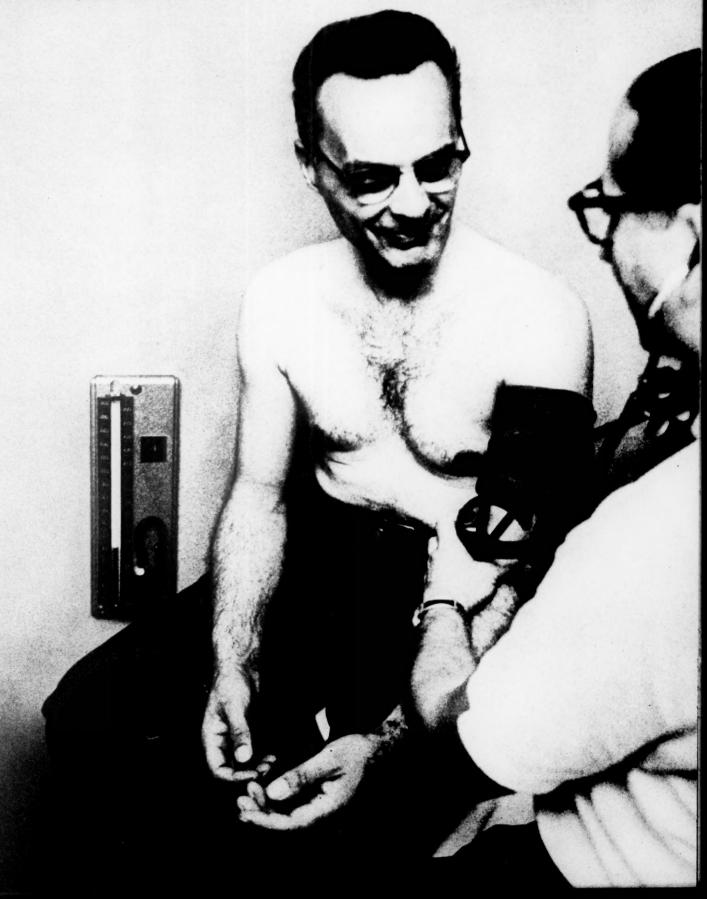


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this hypertensive patient prefers Singoserp

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Before Treatment	190		Whole Root Rauwolfia			Singoserp		
		100	Side Effects: extreme nasal stuffiness headache disturbed sleep	140		No Side Effects	130	
	ပ	.c	apprehensiveness	O	. <u>:</u>		O	80 . <u></u>
	Systolic	Diastolic		Systolic	Diastolic		Systolic	Diastolic

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	Orthuse (gre	ims (day)
6/12/57	2.0	55:
8/7/57	1.0	65
10/2/57	1.0	55
11/29/57	1.0	65
1/17/58	3.0	555
2/14/58	3.0	として
3/28/58	2.0	35
5/5/58	1.5	000

Actual doses used to maintain optimum control in patient J.S., male, age 541

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Coolidge, C. W.; Glisson, C. S., and Smith, A. S.; J.M.A. Georgia 48:167, 1959.

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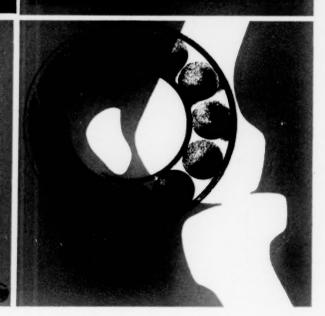
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sustained
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Chlophedianol HCI

cough suppressant action

equal to

narcotics

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duration of action

greater than

narcotics

side actions less than

narcotics

presenting many other advantages

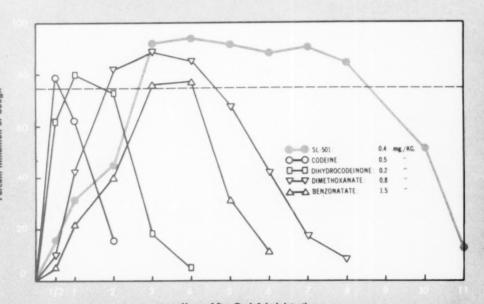




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A comparison of ULO (SL-501) and other antitussive agents in inhibiting experimental coughs in animals

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Chen, J. Y.; Biller, H. F., and Montgomery, E. G., Jr.: J. Pharmacol. & Exper. Therap. 128:384, 1960.

CLINICAL RESULTS WITH ULO

in 1078 patients observed by 50 U.S. investigators, 46 of whom were chest physicians

		Results						
Diagnostic Category	Number of Patients	Good to Excellent	Fair	Poor	Not Specified			
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Bronchitis	398	309	42	38	9			
Pneumonia	53	44	4	5	0			
Postnasal Drip	48	32	9	3	4			
Tracheobronchitis	32	23	4	3	2			
Croup	14	10	2	2	0			
Pleurisy	12	11	0	1	0			
Total Patients	1078	786	149	109	34			
Total Patients Benefited			86.2%					

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required.

ULO Syrup, 25 mg. per teaspoonful, in bottles of 12 fluid ounces.





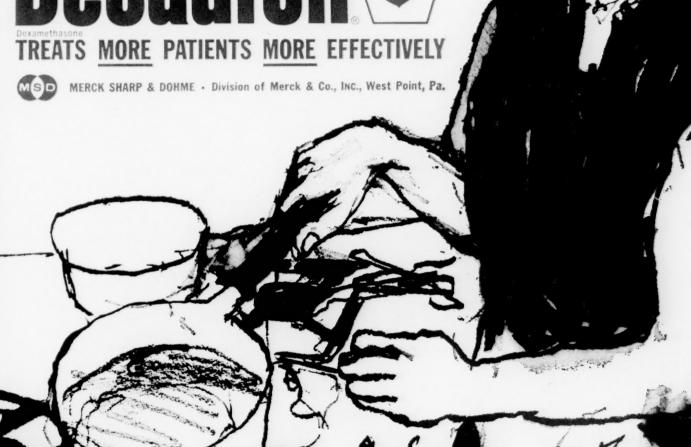
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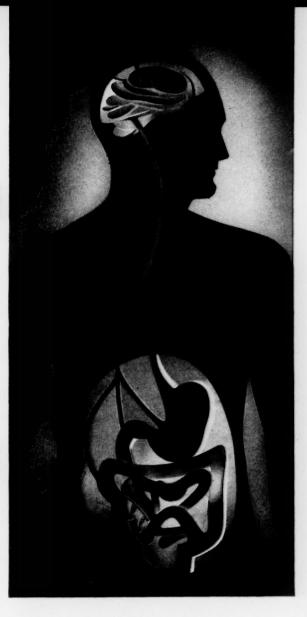
Volume I: General Considerations, is designed to give the student and general practitioner the basic methods of diagnosis of congenital malformations of the heart, and of the care of patients. 63 illustrations.

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50 mg. HydroDIURIL, 0.125 mg. reserpine, 572 mg. potassium chloride. One tablet one or two times a day.

If the patient is receiving ganglion blocking drugs or hydralazine, their dosage must be cut in half when HYDROPRES is added.

For additional information, write Professional Services, Merck Sharp & Dohme, West Point, Pa.



MERCK SHARP & DOHME, DIVISION OF MERCK & CO., INC., WEST POINT, PA.

*HYDROPRES, HYDROPRES-Ka, AND HYDRODIURIL ARE TRADEMARKS OF MERCK & CO., INC.

In depression

To restore emotional stability during the declining years



Tofrānil

brand of imipramine hydrochloride

Thymoleptic

New

for geriatric use

Tablets of 10 mg.

Recent studies 1-3 strongly indicate underlying depression as a causative factor, and Tofranil as an eminently successful agent, in restoring the difficult geriatric patient to a more contented frame of mind and more manageable disposition.

1. Cameron, E.: The Use of Tofranil in the Aged, Canad. Psychiat. A. J. Special Supplement, 4:S160, 1959. 2. Christe, P.: Indications for Tofranil in Geriatrics, Schweiz. med. Wchnschr. 90:586, 1960. 3. Schmied, J., and Ziegler, A.: Tofranil in Geriatrics, Praxis 49:472, 1960.

Also Available:

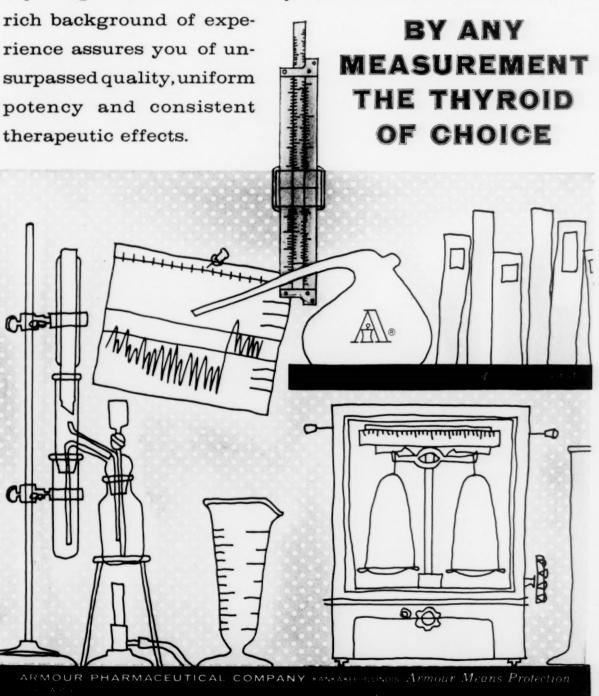
For the treatment of non-geriatric depression: Tofrānil tablets of 25 mg. and ampuls of 25 mg. in 2 cc. solution.

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Oxyphencyclimine hydrochlo

IN BRIEF

DARICON, oxyphencyclimine hydrochloride, is a long-acting highly effective anticholinergic providing potent antispasmodic and antisecretory actions. Each white, scored tablet contains 10 mg. of oxyphencyclimine hydrochloride. DARICON provides 24 hour relief from the pain and discomfort of peptic ulcers, usually with just b.i.d. dosage. A high percentage of refractory ulcer cases have responded well to DARICON.

INDICATIONS: DARICON is valuable for the adjunctive management of peptic ulcers—duodenal, gastric and marginal types; functional bowel syndrome—irritable colon, spastic colon including mucous colitis; pylorospasm, cardiospasm; chronic nonspecific ulcerative colitis; biliary tract disease, including cholecystitis and cholelithiasis; hiatus hernia accompanied by esophagitis or ulcer; gastritis, acute or hypertrophic; duodenitis; bladder spasm with or without cystitis; ureteral spasm, as with stones or pyelonephritis.

SIDE EFFECTS & PRECAUTIONS: Dryness of the mouth is the most common peripheral effect. Blurring of vision, constipation, and urinary hesitancy or retention occur infrequently. These effects may decrease or disappear as therapy continues, or can be minimized by adjustment of dosage. Caution should be exercised when using DARICON in patients with prostatic hypertrophy or glaucoma.

ADMINISTRATION & DOSAGE: The average adult dosage is 10 mg. of DARICON given twice daily — in the morning and at night before retiring. (Dosage should be adjusted in relation to therapeutic response.) As much as 50 mg. daily is acceptable to some adult patients. As little as 5 mg. daily is therapeutically effective in some adult patients.

SUPPLY: DARICON is supplied as a white scored 10 mg. tablet.

More detailed professional information available on request.

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It is important, in the over-all management of duodenal ulcers, that the healing process established during the waking hours continue uninterrupted during the sleeping hours.

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> Kestler, O.: Conservative Management of "Low Back Syndrome", J.A.M.A. 172: 2039 (April 30) 1960.

FASTER IMPROVEMENT-79% complete or marked improvement in 7 days (Kestler).

EASY TO USE—Usual adult dose is one 350 mg. tablet three times daily and at bedtime.

SUPPLIED: 350 mg., white tablets, bottles of 50. For pediatric use, 250 mg., orange capsules, bottles of 50. Literature and samples on request.



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Prompt, Profound Protection...at both ends of the vagus

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Professional reliance on the therapeutic proficiency of Pro-Banthīne in functional gastro-intestinal disorders has made it the most widely prescribed anticholinergic.

The consistent relief of emotional tensions afforded by Dartal makes this well-tolerated tranquilizer a rational choice to support the antispasmodic action of Pro-Banthīne in emotionally influenced smooth-muscle spasm.

These two reliable agents combined as Pro-Banthīne with Dartal consistently control both disturbed mood and disordered motility when emotional disturbances project themselves through the vagus to provoke such gastrointestinal dysfunctions as gastritis, pylorospasm, peptic ulcer, spastic colon or biliary dyskinesia.

USUAL ADULT DOSAGE:

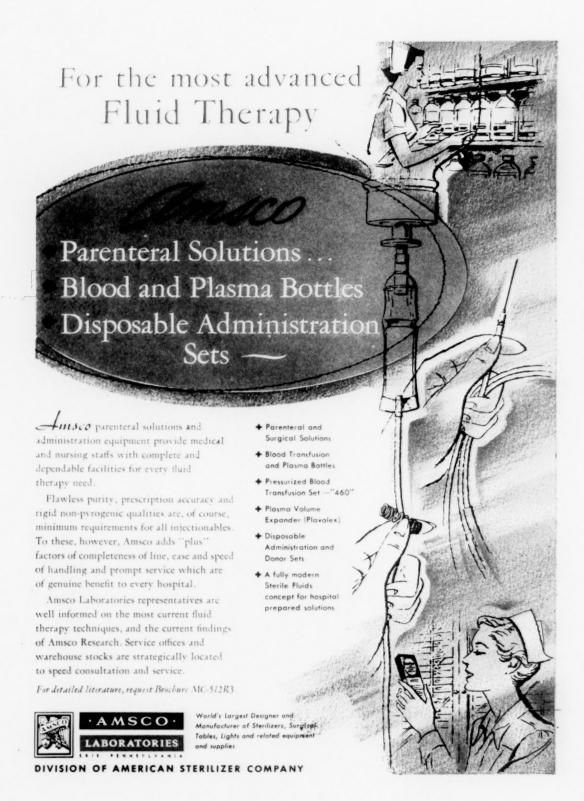
One tablet three times a day.

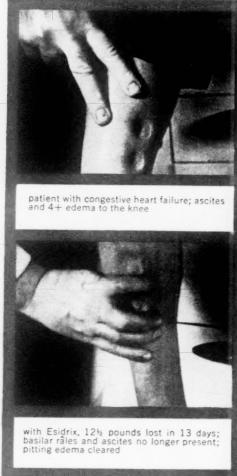
SUPPLIED as aqua-colored, compression-coated tablets containing 15 mg. of Pro-Banthine (brand of propantheline bromide) and 5 mg. of Dartal (brand of thiopropazate dihydrochloride).

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benefits in edema, benefits in hypertension plus built-in potassium protection

NEW ESIDRIX-K

New ESIDRIX-K provides all the oral diuretic-antihypertensive advantages of ESIDRIX, plus a generous potassium supplement. ESIDRIX produces marked excretion of salt and water in edematous patients, and in many hypertensive patients significantly reduces blood pressure, alone or with other antihypertensive drugs. Potassium excretion is minimal, and the built-in K supplement further helps eliminate problems due to potassium loss. Three ESIDRIX-K tablets provide potassium equivalent to one quart of fresh orange juice; ESIDRIX-K is coated to prevent gastric irritation.

Complete information sent on request.

Supplied: Esidrix-K <u>Tablets</u> (white, coated), each containing 25 mg. Esidrix and 500 mg. potassium chloride. Esidrix <u>Tablets</u>, 25 mg. (pink, scored) and 50 mg. (yellow, scored).

Esidrix-K is especially indicated for patients in whom even moderate potassium loss can cause complications, or those whose condition predisposes to hypokalemia. Among candidates for Esidrix-K are patients taking digitalis for congestive heart failure, those with renal or liver disease, those under long-term treatment, and those on salt-restricted diets.



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Aristocort Triamcinolone has long since proved its unsurpassed efficacy and relative safety in the therapy of rheumatoid arthritis, inflammatory and allergic dermatoses, bronchial asthma, and all other conditions in which corticosteroids are indicated. But ARISTOCORT has also opened up new areas of therapy for selected patients who otherwise could not be given corticosteroids. Medicine is now in an era of "special-purpose" steroids.1

One outstanding advantage of triamcinolone is that it rarely produces edema and sodium retention.1,2

The clinical importance of this property cannot be overemphasized in treating certain types of patients. McGavack and associates3 have reported the beneficial results with ARISTOCORT in patients with existing or impending cardiac failure, and those with obesity associated with lymphedema. Triamcinolone, in contrast to most other steroids, is not contraindicated in the presence of edema or impending cardiac decompensation.3

Hollander points out the superiority of triamcinolone in not causing mental stimulation, increased appetite and weight gain, compared to other steroids which produce these effects in varying degrees. And McGavack,2 in a comparative tabulation of steroid side effects, indicates that triamcinolone does not produce the increased appetite, insomnia, and psychic disturbances associated with other newer steroids.

ARISTOCORT can thus be advantageous for patients requiring corticosteroids whose appetites should not be stimulated, and for those who are already overweight or should not gain weight. Likewise, ARISTOCORT is suitable for the many patients with emotional and nervous disorders who should not be subjected to psychic stimulation. Furthermore, ARISTOCORT Triamcinolone, in effective doses, showed a low incidence of side reactions and is a steroid of choice for treating the older patient in whom salt and water retention may cause serious damage.2

References: 1. Hollander, J. L.: J.A.M.A. 172:306 (Jan. 23) 1960. 2. McGavack, T. H.: Nebraska M. J. 44:377 (Aug.) 1959. 3. McGavack, T. H.; Kao, K. Y. T.; Leake, D. A.; Bauer, H. G., and Berger, H. E.; Am. J. M. Sc. 236:720 (Dec.) 1958.

Precautions: Collateral hormonal effects generally associated with corticosteroids may be induced. These include Cushingoid manifestations and muscle weakness. However, sodium and potassium retention, edema, weight gain. psychic aberration and hypertension are exceedingly rare. Dosage should be individualized and kept at the lowest level needed to control symptoms. It should not exceed 36 mg. daily without potassium supplementation. Drug should not be withdrawn abruptly. Contraindicated in herpes simplex and

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Alcohol

Supplied: Bottles of 16 ounces and 1 gallon.

Dosage: Every three or four hours—adults, 1 to 2 teaspoonfuls; children ½ to 1 teaspoonful.

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In an extensive clinical study* involving 109 children with petit mal, the investigators found ZARONTIN to be: EFFECTIVE —"Quite a few patients, never before helped by any drug, have been completely con-

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See medical brochure, available to physicians, for details of administration and dosage. *Zimmerman, F. T., & Burgemeister, B. B.: Neurology 8:769, 1958.

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Triacetyloleandomycin, equivalent to oleandomycin 125 mg. This is the URI antibiotic, clinically effective against certain antibiotic-resistant organisms.

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Triaminic[®], 25 mg., three active components stop running noses. Relief starts in minutes, lasts for hours.

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Calurin®, calcium acetylsalicylate carbamide equivalent to aspirin 300 mg. This is the freely-soluble calcium aspirin that minimizes local irritation, chemical erosion, gastric damage. High, fast blood levels.

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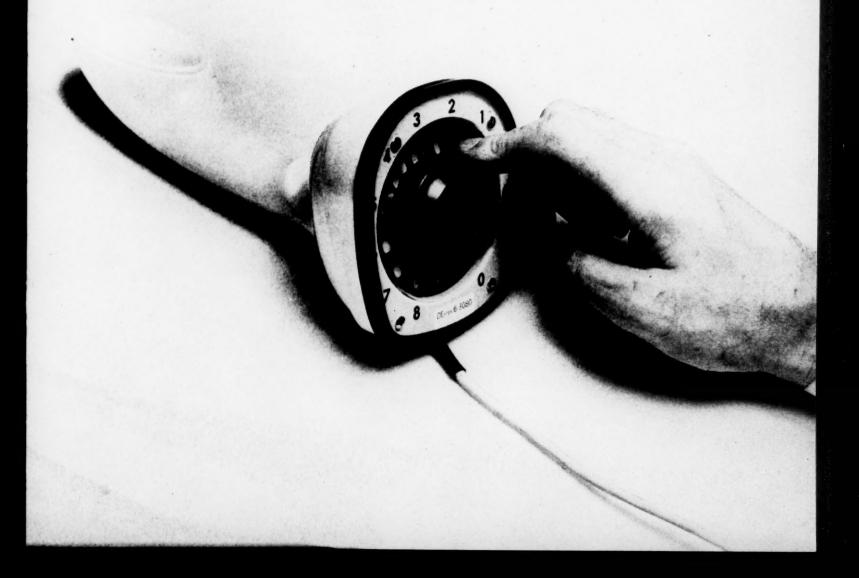
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Longest acting of any available thiazide, more sodium excretion with less potassium loss





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an improved way to treat edema and hypertension

once a day, every day

If you've found the thiazide diuretics helpful, you'll particularly appreciate Enduron. It provides the familiar benefits of oral thiazide therapy, but in a new and (we feel) more practical manner. Dosage, for example, is engineered for the most practical schedule of all: "Once a day, every day." Easy to remember, easy to stick to. More important, duration of action of this single daily dose is over 24 full hours. This means your first dose is still producing good diuresis or hypotensive action right up to the time when the next day's dose takes effect.

A single dose of 10 mg. produces a peak natruretic effect. By this we mean that the maximum possible effect occurs with 10 mg., and greater doses do not produce greater natruresis. However, most patients require just 5 mg. daily for satisfactory response. Some can be maintained on as little as 2½ mg. Such small doses afford a very safe therapeutic ratio. ■ If you're concerned about potassium, too, you'll like Enduron. It produces the least potassium excretion of any available thiazide. Depletion seldom becomes a factor in your therapy.

See next page for more details . . .

ENDURON

(METHYCLOTHIAZIDE, ABBOTT)

logical culmination to thiazide therapy

MOST SUSTAINED ACTION OF ANY AVAILABLE THIAZIDE

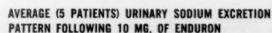
For once a day dosage to be really satisfactory, it must continue to produce therapeutic effect more than 24 hours later. Otherwise you can expect a gap in action until next day's dose has time enough to re-establish the effect. This gap is avoided with Enduron therapy. Its action remains well above control (i.e., undosed) levels, even at the end of the 24-hour period.

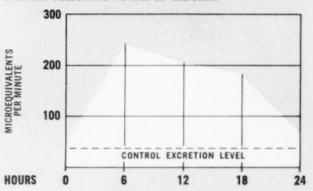


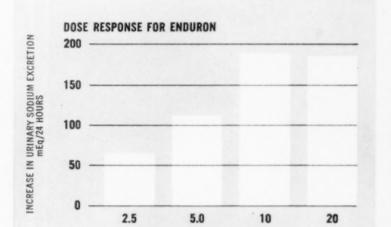
Enduron is about 20 times more potent than hydrochlorothiazide by weight. It is also more potent compared at peak doses. As explained before, by peak doses we mean the smallest amounts which produce maximum natruretic response. In Enduron that peak is achieved with just 10 mg. (see graph). Larger doses than 10 mg. don't produce additional effect, and aren't needed.

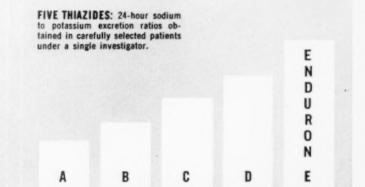
MOST POTASSIUM-SPARING OF ANY AVAILABLE THIAZIDE

Enduron enhances sodium excretion, but doesn't boost potassium outgo proportionately. Its ratio of sodium excretion versus potassium is the most favorable of any available thiazide. In other words, Enduron leads to greater sodium excretion per unit of potassium excreted, and to less total potassium loss than other thiazides. Thus potassium depletion is rarely a problem.









6.0 to 1

7.5 to 1

SINGLE DOSE IN MILLIGRAMS

Enduron indications and side reactions are generally comparable to those for the earlier thiazides. Diuresis is prompt, but like other thiazides, several weeks may be required for full hypotensive effect. Enduron has a potentiating action, and you may wish to adjust dosage of other antihypertensive agents if they are used at the same time. Our literature gives full details; ask any Abbott representative or write Abbott Professional Services, North Chicago, Illinois.

3.2 to 1

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10.5 to 1



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Panmycin (tetracycline) equivalent to tetracycline hydrochloride...... 125 mg. Albamycin (as novobiocin calcium) 62.5 mg. Potassium Metaphosphate 100 mg.

Supplied: In 40 cc. and 60 cc. bottles.

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in potentiallyserious pediatric
infections,
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Panalba KM*Granules

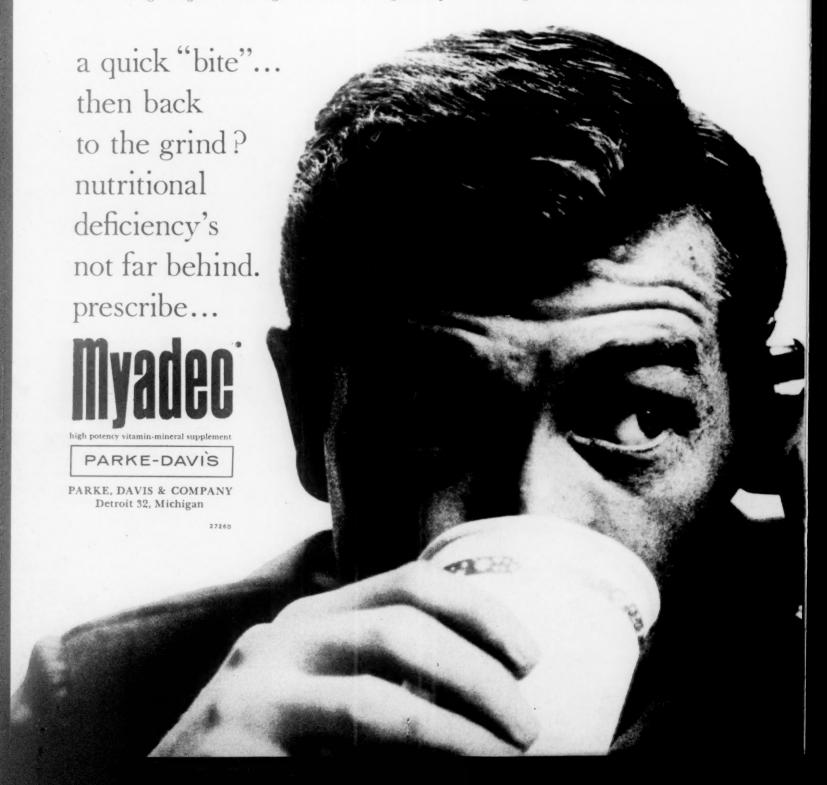
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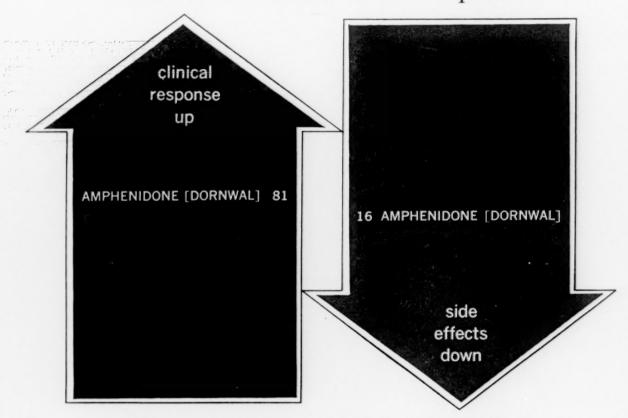
Each Myadec Capsule contains: Vitamins: Vitamin B₁₂ crystalline—5 mcg.; Vitamin B₂ (riboflavin)—10 mg.; Vitamin B₃ (pyridoxine hydrochloride)—2 mg.; Vitamin B₁ mononitrate—10 mg.; Nicotinamide (niacinamide)—100 mg.; Vitamin C (ascorbic acid)—150 mg.; Vitamin A—(7.5 mg.) 25,000 units; Vitamin D—(25 mcg.) 1,000 units; Vitamin E (d-alpha-tocopheryl acetate concentrate)—5 I.U. MINERALS: (as inorganic salts) Iodine—0.15 mg.; Manganese—1 mg.; Cobalt—0.1 mg.; Potassium—5 mg.; Molybdenum—0.2 mg.; Iron—15 mg.; Copper—1 mg.; Zinc—1.5 mg.; Magnesium—6 mg.; Calcium—105 mg.; Phosphorus—80 mg. Bottles of 30, 100 and 250.



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for greater therapeutic effectiveness in anxiety and tension with lowest incidence of side effects for the outpatient



Indications: Anxiety and tension states, tension headaches, pre- and postoperative apprehension, anxiety coexistent with gastrointestinal, dermatologic, gynecologic, cardiovascular and other functional or organic disorders, behavior disorders in children associated with anxiety and tension.

Dose: Adults, one or two 200 mg. tablets three times a day. Children, 6 to 16, one or two 100 mg. tablets two times a day. Administration limited to three months duration.

Supplied: 200 mg. yellow scored tablets, and 100 mg. pink tablets, each in bottles of 100 and 500.

*Nodine, J. H.; Bodi, T.; Slap, J.; Levy, H. A., and Siegler, P. E.: Human bioassay of tranquilizers in psychosomatic disorders, Scientific Exhibit, American Medical Association Annual Meeting, Miami Beach, Florida, June 13-17, 1960.

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As directed, the patient takes one Meprospan-400 capsule at breakfast. Her symptoms of tension and nervousness are soon relieved, and she will not have to remember to take another capsule until dinnertime.



Calm and relaxed, the patient is no longer upset by the pressures and irritations met in everyday life, nor is she likely to be incapacitated by autonomic disturbances, drowsiness, ataxia or other untoward reactions.



Alert and attentive, the patient participates in a P.T.A. meeting, following her second capsule of Meprospan-400 taken with the evening meal. Meprospan-400 does not decrease her mental efficiency or interfere with her normal activities or behavior.



Peacefully asleep, the patient enjoys beneficial rest... Meprospan-400 has relieved the tensions that previously prevented sleep or kept her tossing and turning throughout the night.

most widely prescribed tranquilizer... most convenient dosage form...

ONE CAPSULE LASTS 12 HOURS

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Usual dosage: One capsule at breakfast lasts all day, one capsule with evening meal lasts all night. Supplied: Meprospan-400, each bluetopped sustained-release capsule contains 400 mg. Miltown. Also available: Meprospan-200, each yellow-topped sustained-release capsule contains 200 mg. Miltown. For children: Capsules can be opened and the coated granules mixed with soft foods or liquids.

Both potencies in bottles of 30.

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Esidrix). Complete information available on request. Singoserp-Esidrix (syrosingopine Pand hydrochlorothiazide CIBA)



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Each capsule contains: Ethinyl Estradiol 0.01 mg. • Methyl Testosterone 2.5 mg. • d-Amphetamine Sulfate 2.5 mg. • Vitamin A (Acetate) 5,000 U.S.P. Units • Vitamin D 500 U.S.P. Units • Vitamin B₁₂ with AUTRINIC® Intrinsic Factor Concentrate 1/15 U.S.P. Unit (Oral) • Thiamine Mononitrate (B₁) 5 mg. • Riboflavin (B₂) 5 mg. • Niacinamide 15 mg. • Pyridoxine HCl (B₄) 0.5 mg. • Calcium Pantothenate 5 mg. • Choline Bitartrate 25 mg. • Inositol 25 mg. • Ascorbic Acid (C) as Calcium Ascor

bate 50 mg. • I-Lysine Monohydrochloride 25 mg. • Vitamin E (Tocopherol Acid Succinate) 10 Int. Units • Rutin 12.5 mg. • Ferrous Fumarate (Elemental iron, 10 mg.) 30.4 mg. • Iodine (as KI) 0.1 mg. • Calcium (as CaHPO $_{\!\!4}$) 35 mg. • Phosphorus (as CaHPO $_{\!\!4}$) 27 mg. • Fluorine (as CaF $_{\!\!2}$) 0.1 mg. • Copper (as CuO) 1 mg. • Potassium (as K $_{\!\!4}\text{SO}_{\!\!4}$) 5 mg. • Manganese (as MnO $_{\!\!2}$) 1 mg. • Zinc (as ZnO) 0.5 mg. • Magnesium (MgO) 1 mg. • Boron (as Na $_{\!\!2}\text{B}_{\!\!4}\text{O}_{\!\!2}$.10H $_{\!\!2}\text{O}$) 0.1 mg. Bottles of 100, 1000.

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Cremomycin, provides rapid relief of virtually all diarrheas

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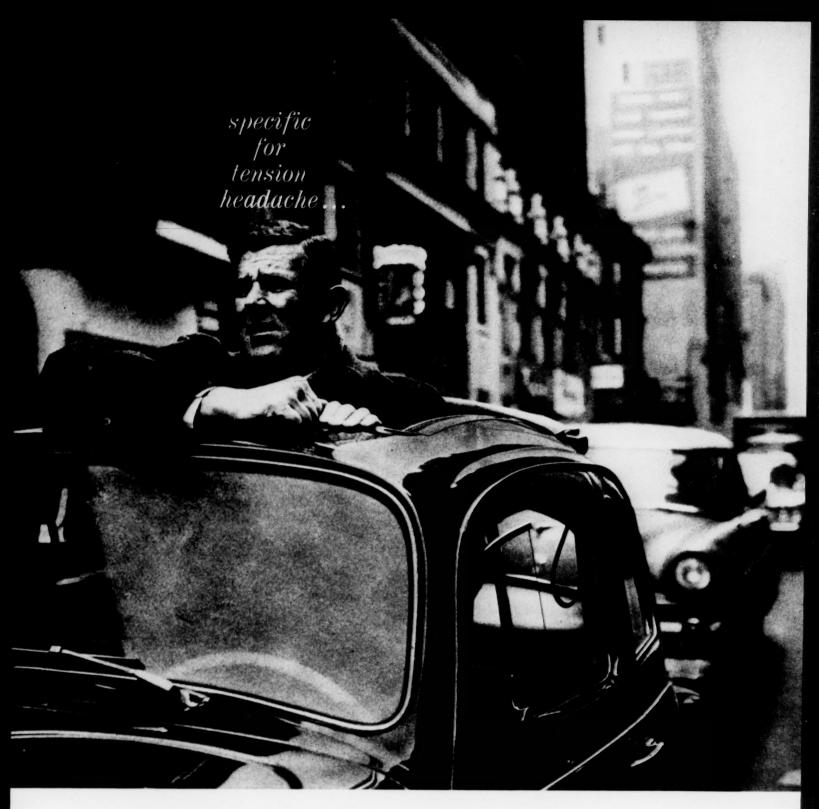
NEOMYCIN -rapidly bactericidal against most intestinal pathogens, but relatively ineffective against certain diarrhea-causing organisms.

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KAOLIN AND PECTIN-coat and soothe the inflamed mucosa, adsorb toxins, help reduce intestinal hypermotility, help provide rapid symptomatic relief.

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HORIVAI relieves pain, muscle sp

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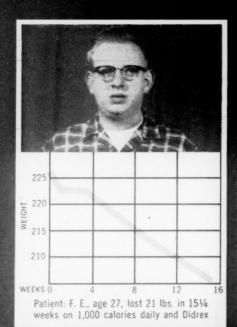
"We have found caffeine, used in combination with acetylsalicylic acid, acetophenetidin, and isobutylallylbarbituric acid, [Fiorinal] to be one of the most effective medicaments for the symptomatic treatment of headache due to tension." Friedman, A. P., and Merritt, H. H.: J.A.M.A. 163:1111 (Mar. 30) 1957.

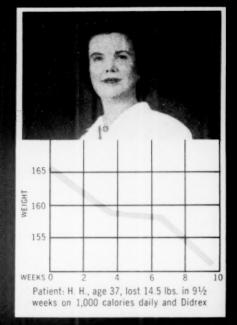
Available: Fiorinal Tablets and New Form — Fiorinal Capsules

Each contains: Sandoptal (Allylbarbituric Acid N.F. X) 50 mg. (3/4 gr.), caffeine 40 mg. (2/3 gr.), acetylsalicylic acid 200 mg. (3 gr.), acetophenetidin 130 mg. (2 gr.).

Dosage: 1 or 2 every four hours, according to need, up to 6 per day.







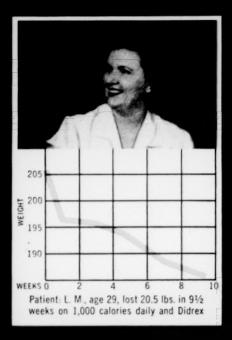


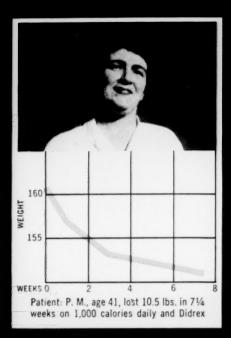
UPJOHN ANNOUNCES

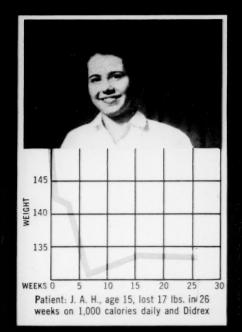


HELPS TAKE WEIGHT OFF... PERSISTENTLY

Didrex







WEEK AFTER WEEK

in obesity management Put it to your patient this way: The basic therapeutic objective of obesity management is to change dietary habits built over

months or years of weight accumulation. This takes time and will. Consider Didrex, the new Upjohn appetite suppressant. Happily, it elevates mood which makes dieting more acceptable. More important, it works: "persistent significant weight loss" in patients followed for as long as 20 weeks. Added to your favorite reducing regimen, ½ to 1 Didrex tablet one to three times daily is usually adequate to preclude the "weight plateau" that so often discourages dieters after a few weeks. Available as 50 mg. tablets in bottles of 100.

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BRIEF BASIC INFORMATION

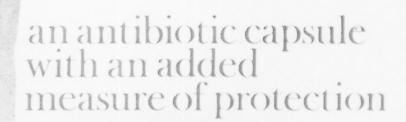
Description: Didrex is the Upjohn brand of benzphetamine hydrochloride $((+)-N-benzyl-N,\alpha-dimethyl-phenethyl$ amine hydrochloridel. A sympathomimetic compound with marked anorexic action and relatively little stimulating effect on the CNS or cardiovascular system.

Indications: Control of obesity.

Contraindications: None known, However, use with caution in moderate or severe hypertension, thyrotoxicosis, acute coronary disease, or cardiac decompensation.

Dosage: Initiate appetite control with 1/2 or 1 tablet (25 to 50 mg.) in midmorning for several days. Then adjust dosage to suit each patient's need to a maximum of 3 tablets daily (150 mg.). Side Effects: No effects on blood, urine. renal or hepatic functions have been noted. Minimal side effects, similar to those reported from placebos, have been observed occasionally: dry mouth, insomnia, nausea, palpitations and nervousness.

Supplied: 50 mg., press-coated, scored tablets, in bottles of 100.



AGAINST RELAPSE-up to 6 days' activity with 4 days' dosage

AGAINST "PROBLEM" PATHOGENS - uniformly sustained peak activity

AGAINST: SECONDARY INFECTION - full antibiotic

DISTINCTIVE, DRY-FILLED, DUOTONE RED CAPSULES -

150 mg., bottles of 16 and 100. Dosage: 1 capsule (150 mg.)

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he'll be fitter sooner when you prescribe

(Paraflex's + Tylenol*)

Prescribe PARAFON (2 tablets t.i.d. or q.i.d.) in sprains—strains—myositis—whiplash injuries—low back pain

Each Parafox tablet contains:

Paraflex* Chlorzoxazone* 125 mg. Specific for skeletal muscle spasm Tylfnol.* Acetaminophen 300 mg. Superior analgesic in musculoskeletal pain

and in arthritis

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PARALLEX* 125 mg., TYLENOL* 300 mg., and prednisolone 1.0 mg.

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†U.S. Patent No. 2,895,877

McNeil Laboratories, Inc. Philadelphia 32, Pa.





Introducing ...

Miluretic*

new therapy for hypertension and congestive failure

For samples and complete literature, write to

lowers blood pressure drains excess water calms apprehension

Created especially for those patients whose emotional condition complicates the treatment of hypertension and congestive failure

Now the most widely prescribed diuretic-antihypertensive, hydro-chlorothiazide, is combined with the most widely prescribed tranquilizer, meprobamate. Called "Miluretic", it constitutes new, effective therapy for hypertension and congestive failure—especially when emotional factors complicate your treatment.

What does Miluretic do? Both components are of proven value in hypertension. And in congestive failure, Miluretic induces smooth, continuous diuresis. Miluretic's biggest advantage is that it tranquilizes hypertensive and edematous patients safely and quickly.

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Antihypertensive agents derived from Rauwolfia often cause reactions such as depression and nasal congestion; Miluretic does not.

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MILTOWN + HYDROCHLOROTHIAZID

Available at all pharmacies Composition: 200 mg. Miltown (meprobamate, Wallace) + 25 mg. hydrochlorothiazide

Dosage: For hypertension, 1 tablet four times a day. For congestive failure, 2 tablets four times a day.

Supplied: Bottles of 50 white, scored tablets

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- more patients are receiving the benefits of
 - more clinical evidence exists for —



"Chlorothiazide was given to 16 patients for a total of 295 patient-treatment days." "Chlorothiazide is a safe, oral diuretic with a clinical effect equal to or greater than a parenteral mercurial." Harvey, S. D. and DeGraff, A. C.: N. Y. State J. Med., **59**:1769, (May 1) 1959.



"... our program has been one of polypharmacy in which we attempt to deplete body sodium with chlorothiazide. This drug is continued indefinitely as background medication for all antihypertensive drugs." Moyer, J. H.: Am. J. Cardiology, 3:199, (Feb.) 1959.



"Chlorothiazide is an excellent agent for relief of swelling and breast soreness associated with the premenstrual tension syndrome, since all patients [50] with these complaints were completely relieved." Keyes, J. W. and Berlacher, F. J.: J.A.M.A., 169:109, (Jan. 10) 1959.

DOSAGE: Edema—One or two 500 mg. tablets DIURIL once or twice a day. Hypertension—One 250 mg. tablet DIURIL twice a day to one 500 mg. tablet DIURIL three times a day.

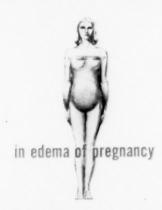
SUPPLIED: 250 mg. and 500 mg. scored tablets DIURIL (chlorothiazide) in bottles of 100 and 1,000.

DIURIL is a trademark of Merck & Co., INC.

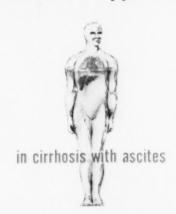
Additional information is available to the physician on request.

hynertension

than for all other diuretic-antihypertensives combined!



"One hundred patients were treated with oral chlorothiazide." "In the presence of clinically detectable edema, the agent was universally effective." "Chlorothiazide is at present the most effective oral diuretic in pregnancy." Landesman, R., Ollstein, R. N. and Quinton, E. J.: N. Y. State J. Med., 59:66, (Jan. 1) 1959.



"All three of the patients with Laennec's cirrhosis, ascites and edema had a favorable response, with a mean weight loss of 8 lbs., during the fiveday treatment period with a slight decrease in edema." Castle, C. N., Conrad, J. K. and Hecht, H. H.: Arch. Int. Med., 103:415, (March) 1959.



"In a study of 10 patients with the nephrotic syndrome associated with various types of renal disease, orally administered chlorothiazide was a successful, and sometimes dramatic, diuretic agent." Burch, G. E. and White, M. A., Jr.: Arch. Int. Med., 103:369, (March) 1959.



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without whipping the bowel

DORBANE



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DORDANTYL FORTE

The active principle of Dorbane reaches the colon through the circulation. It acts directly and selectively upon the intrinsic plexus of the colon. The small bowel is not affected. Within 6 to 12 hours evacuation occurs without cramping or griping. Non-habituating. Each scored tablet of Dorbane contains 75 mg., and each teaspoonful of orange-flavored liquid contains 37.5 mg. of 1,8 dihydroxyanthraquinone. Suitable for patients of all ages.

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Dorbantyl Forte offers double strength dosage of the Dorbantyl combination for greater convenience and economy for patients requiring extra potency. In orange-and-gray capsules only.



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NEW

RAPID SCREENING TEST FOR

HYPOFIBRINOGENEMIA

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CONTROL

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FI-TEST*

Test results at patient's bedside – from skin puncture to reading – in less than 2 minutes. Only one drop of blood required. Test performed by simple, rapid-slide technic.

FI-TEST indicates whether fibrinogen content is above or below 100 mg-%, the concentration considered critical. Easy-to-read results indicate promptly whether or not replacement fibrinogen is needed. (If reading shows a normal fibrinogen level, needless replacement therapy may be avoided and the physician is alerted to seek another explanation for continued bleeding.)

Supplied in compact ready-to-use kits containing complete materials for 6 determinations.



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both are free of pain-but only one is on

DILAUDI

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swift, sure analgesia normally unmarred by nausea and vomiting

DILAUDID provides unexcelled analgesia in acute cardiovascular conditions. Onset of relief from pain is almost immediate. The high therapeutic ratio of DILAUDID is commonly reflected by lack of nausea and vomiting—and marked freedom from other side-effects such as dizziness and somnolence.

> by mouth by needle by rectum

> > 2 mg., 3 mg., and 4 mg.

May be habit forming—usual precautions should be observed as with other opiate analgesics.



KNOLL PHARMACEUTICAL COMPANY · ORANGE, NEW JERSEY



IN ORAL CONTROL OF PAIN

ACTS FASTER—usually within 5-15 minutes. LASTS LONGER—usually 6 hours or more. MORE THOROUGH RELIEF—permits uninterrupted sleep through the night. RARELY CONSTIPATES—excellent for chronic or bedridden patients.

AVERAGE ADULT DOSE: 1 tablet every 6 hours. May be habit forming. Federal law permits oral prescription.

Each Percodan* Tablet contains 4.50 mg. dihydrohydroxycodeinone hydrochloride, 0.38 mg. dihydrohydroxycodeinone terephthalate, 0.38 mg. homatropine terephthalate, 224 mg. acetylsalicylic acid, 160 mg. phenacetin, and 32 mg. caffeine.

Also available — for greater flexibility in dosage — Percodan "-Demi: The Percodan formula with one-half the amount of salts of dihydrohydroxycodeinone and homatropine.



5

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FOR PALLY



the complaint: "nervous indigestion"

the diagnosis: any of several nonspecific and functional in the gastric-soluble outer layer: gastrointestinal disorders requiring relief of symptoms by sedative-antispasmodic action with concomitant digestive enzyme therapy.

the prescription: a new formulation incorporated in an enteric-coated tablet, providing the multiple actions of widely accepted Donnatal® and Entozyme.®

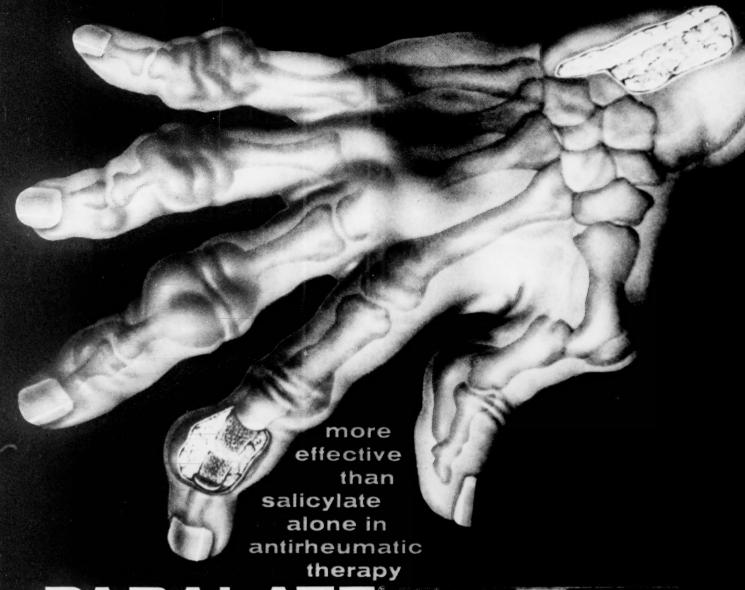
the dosage: two tablets three times a day, or as indicated.

6	
Hyoscyamine sulfate0.0518 mg.	
Atropine sulfate 0.0097 mg.	
Hyoscine hydrobromide0.0033 mg.	
Phenobarbital (1/8 gr.)8.1 mg.	
Pepsin, N. F	

in the enteric-coated core:

Panc	reatin,	N.	F.												300	mg.
Bile	salts.									•					150	mg.

antispasmodic · sedative · digestant



PABALATE

Robins

COMBINING MUTUALLY SYNERGISTIC NON-STEROID ANTIRHEUMATICS

"superior to aspirin" — "... evidence seems to indicate that the concurrent administration of para-aminobenzoic and salicylic acid [as in Pabalate] produces a more uniformly sustained level for prolonged analgesia and, therefore, is superior to aspirin in the treatment of chronic rheumatic disorders."

In each yellow enteric-coated PABALATE tablet:

Sodium salicylate (5 gr.)	0.3	Gm.
Sodium para-aminobenzoate (5 gr.)	0.3	Gm.
Ascorbic acid	50.0	mg.

For the patient who should avoid sodium

PABALATE-SODIUM FREE

Same formula as Pabalate, with sodium salts replaced by potassium salts (pink)

For the patient who requires steroids

PABALATE-HC

Pabalate with Hydrocortisone

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Hydrocortisone	2.5 mg.
Potassium salicylate (5 gr.)	0.3 Gm.
Potassium para-aminobenzoate (5 gr.)	0.3 Gm.
Ascorbic acid	50 0 mg

1. Ford, R. A., and Blanchard, K.: Journal-Lancet 78:185, 1958.

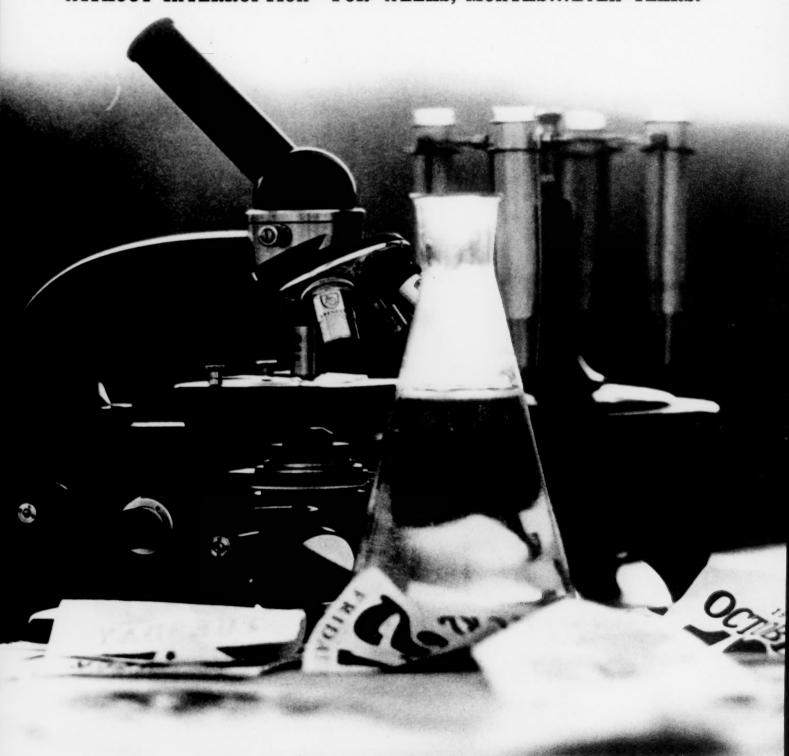
A. H. ROBINS CO., INC., Richmond 20, Virginia







THE SULFA COMPOUND THAT IS ESPECIALLY VALUABLE IN URINARY TRACT INFECTIONS BECAUSE IT CAN BE GIVEN SAFELY-WITHOUT INTERRUPTION — FOR WEEKS, MONTHS...EVEN YEARS.



"Thiosulfil" Forte

See over for therapy in difficult patients ▶

HOW TO IMPROVE THE PROGNOSIS IN THE DIFFICULT PATIENT WITH URINARY TRACT INFECTION: Proof of effectiveness and record of safety in long term therapy are two important factors in the selection of a sulfa, particularly when the infection is stubborn and recurrent; occurs during pregnancy; in prostatitis; in patients with indwelling catheters; when stasis is a potential cause of ascending infection. "Thiosulfil" Forte is specially valuable in the treatment of problem patients with urinary tract infection as demonstrated by years of clinical experience.

RECORD OF SAFETY

In clinical studies of over 3,600 patients, the number of reactions, none serious, was less than 2 per cent.1-6 " 'Thiosulfil' was remarkably well tolerated, there being no discontinuation of treatment due to untoward effects, and very few mild reactions were noted."2 "The drug can be taken over a long period of time with practically no untoward side reactions."3 "Clinical trial appears to indicate that the drug can be tolerated where other sulfa drugs cannot and that it is effective where some others are not." Out of 3,057 cases . . . 47 patients (1.6%) showed g.i. disturbances and 33 patients (1.1%) allergic reactions.1 NO RE-PORTS OF: hemorrhagic dyscrasias, hematuria, anuria, agranulocytosis. . .

PROOF OF EFFECTIVENESS

A review of more than 3,600 reported cases on "Thiosulfil" demonstrates: a) adequate drug dosage can be simply and economically achieved with a minimum incidence of complicating side effects; b) the antibacterial agent can be given over longer periods of time, particularly in cases involving urinary stasis.

Specific For Urinary Tract Infections: "Thiosulfil" reaches greater urinary concentrations in the active, free, nonmetabolized form than any other sulfa, single or mixed. "Thiosulfil" is rapidly excreted; as much as 79% of the drug is found in the urine after eight hours—of this, 98% is in the active form. The entire g.u. tract is, thus, subjected to continual "sulfa baths" of active drug—more wide spectrum antibacterial activity at site of infection.

Even where urinary stasis exists and cannot be readily corrected, prolonged or even indefinite use of "Thiosulfil" on a reduced dosage schedule will usually keep the infection under control, patients comfortable, and side effects minimal. "Thiosulfil" may materially reduce the likelihood of infections ascending to the parenchyma of the kidneys and subsequent serious systemic involvement.

DOSAGE (Urinary Tract Infections)

TIME PERIOD	DOSE
First two weeks	3 Gm./day ¹
2 weeks to 3 months	2 Gm./day ^{2, 6}
3 months or longer	0.5 Gm./day ⁷

Suggested Range of Dosage: 1 or 2 tablets three or four times daily. Note: The usual precautions exercised with sulfonamides should be observed.

Supplied: No. 786: Each tablet contains 0.5 Gm. sulfamethizole; in bottles of 100 and 1,000 scored tablets.

References—1. Bourque, J-P., and Gauthier, G-E.: Seven years' experience with sulfamethizole, to be published. 2. Bourque, J-P., and Joyal, J.: Canad. M.A.J. 68:337 (Apr.) 1953. 3. Barnes, R. W.: J. Urol. 71:655 (May) 1954. 4. Goodhope, C. D.: J. Urol. 72:552 (Sept.) 1954. 5. Boger, W. P.: The Antibacterial Sulfonamides: Comparative Studies, Scientific Exhibit Section, American Academy of General Practice Eleventh Annual Scientific Assembly, Apr. 6-9, 1959, San Francisco, California. 6. Cottrell, T. L. C., Rolnick, D., and Lloyd, F. A.: Rocky Mountain M. J. 56:66 (Mar.) 1959. 7. Hughes, J., Coppridge, W. M., and Roberts, L. C.: North Carolina M. J. 17:320 (July) 1956.

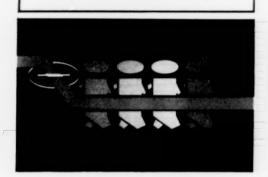
THE SULFA COMPOUND THAT IS ESPECIALLY VAL-UABLE IN URINARY TRACT INFECTIONS BECAUSE IT CAN BE GIVEN SAFELY—WITHOUT INTERRUP-TION—FOR WEEKS, MONTHS...EVEN YEARS.

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IN BRIEF

DIABINESE, a potent sulfonylurea, provides smooth, longlasting control of blood sugar permitting economy and simplicity of low, once-a-day dosage. Moreover, DIABINESE often works where other agents have failed to give satisfactory control.

INDICATIONS: Uncomplicated diabetes mellitus of stable, mild or moderately severe nonketotic, maturity-onset type. Certain "brittle" patients may be helped to smoother control with reduced insulin requirements.

ADMINISTRATION AND DOSAGE: Familiarity with criteria for patient selection, continued close medical supervision, and observance by the patient of good dietary and hygienic habits are essential.

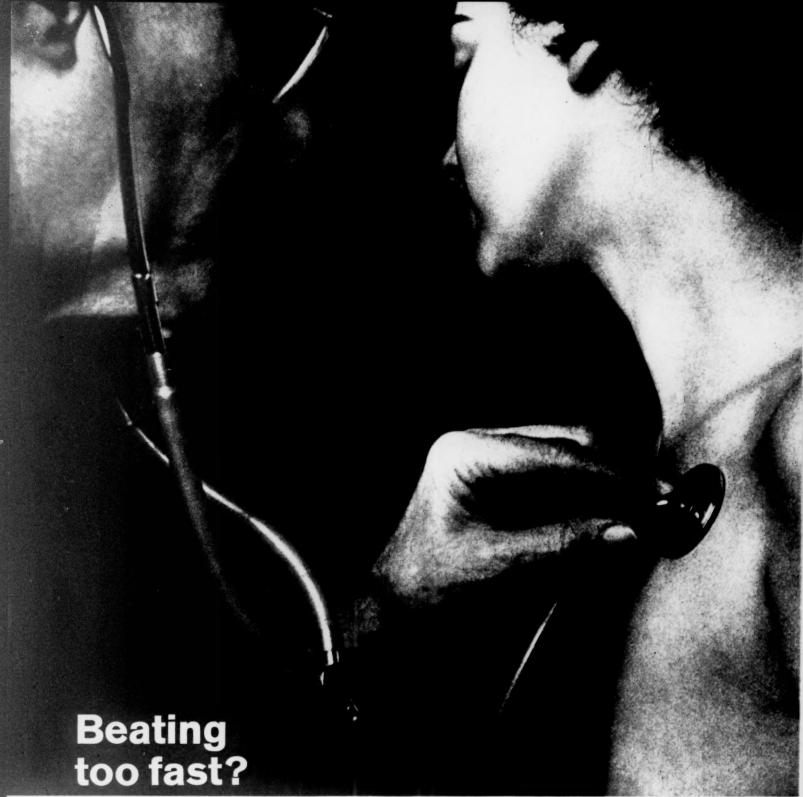
Average maintenance dosage is 100-500 mg. daily. For most patients the recommended starting dose is 250 mg. given once daily. Geriatric patients should be started on 100-125 mg. daily. A priming dose is not necessary and should not be used: most patients should be maintained on 500 mg. or less daily. Maintenance dosage above 750 mg. should be avoided. Before initiating therapy, consult complete dosage information.

SIDE EFFECTS: In the main, side effects, e.g., hypoglycemia, gastrointestinal intolerance, and neurologic reactions, are related to dosage. They are not encountered frequently on presently recommended low dosage. There have been, however, occasional cases of jaundice and skin eruptions primarily due to drug sensitivity: other side effects which may be idiosyncratic are occasional diarrhea (sometimes sanguineous) and hematologic reactions. Since sensitivity reactions usually occur within the first six weeks of therapy, a time when the patient is under very close supervision, they may be readily detected. Should sensitivity reactions be detected, DIABINESE should be discontinued.

PRECAUTIONS AND CONTRAINDICATIONS: If hypoglycemia is encountered, the patient must be observed and treated continuously as necessary, usually 3-5 days, since DIABINESE is not significantly metabolized and is excreted slowly. DIABINESE as the sole agent is not indicated in juvenile diabetes mellitus and unstable or severely "brittle" diabetes mellitus of the adult type. Contraindicated in patients with hepatic dysfunction and in diabetes complicated by ketosis, acidosis, diabetic coma, fever, severe trauma, gangrene, Raynaud's disease, or severe impairment of renal or thyroid function. DIABINESE may prolong the activity of barbiturates. An effect like that of disulfiram has been noted when patients on DIABINESE drink alcoholic beverages.

SUPPLIED: As 100 mg. and 250 mg. scored chlorpropamide tablets.

More detailed professional information available on request.



Slow it down with SERPASIL

Serpasil has proved effective as a heart-slowing agent in the (reserpine CIBA) following conditions: mitral disease; myocardial infarction;

cardiac arrhythmias; neurocirculatory asthenia; thyroid toxicosis; excitement and effort syndromes; cardiac neurosis; congestive failure. Serpasil should be used with caution in patients receiving digitalis and quinidine. It is not indicated in cases of aortic insufficiency.

SUPPLIED: Tablets, 0.1 mg., 0.25 mg. (scored) and 1 mg. (scored). Complete information available on request.

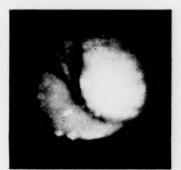
C I B A

Excellent results in ulcerative colitis even where other steroids have failed

Proctoscopic view of the signioid in acute stage of ulcerative colitis



Proctoscopic view of the sigmoid following Depo-Medrol retention enemas for acute stage of ulcerative colitis



Proctoscopic view of sigmoid colon in a normal person

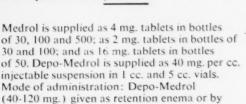


In controlling ulcerative colitis (recurrent, moderately severe, severe, and resistant), Depo-Medrol† can be given topically (by enema or rectal instillation) in requisitely large doses without producing significant side effects. Excellent results are obtainable even where other steroids have failed and improvement continues on oral Medrol maintenance dosage.

there is only one methylprednisolone, and that is

Medrol*

that hits the disease, but spares the patient



^{*}Trademark, Reg. U. S. Pat. Off. - methylprednisolone, Upjohn

continuous drip three to seven times weekly.



in a more acid-stable form assure adequate absorption even when taken with food

Ilosone retains 97.3 percent of its antibacterial activity after exposure to gastric juice (ph 1.1) for forty minutes. This means there is more antibiotic available for absorption—greater therapeutic activity. Clinically, too, Ilosone has been shown^{2.3} to be decisively effective in a wide variety of bacterial infections—with a reassuring record of safety.⁴

Usual dosage for adults and for children over fifty pounds is 250 mg. every six hours. Supplied in 125 and 250-mg. Pulvules and in suspension and drops.

- 1. Stephens, V. C., et al.: J. Am. Pharm. A. (Scient. Ed.), 48:620, 1959.
- 2. Salitsky, S., et al.: Antibiotics Annual, p. 893, 1959-1960.
- 3. Reichelderfer, T. E., et al.: Antibiotics Annual, p. 899, 1959-1960.
- 4. Kuder, H. V.: Clin. Pharmacol. & Therap., in press.

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Editorial

Influenza Viral Pneumonia of Human Beings

SHORTLY after the discovery of influenza virus in 1933 it was found that the agent could induce a pure viral pneumonia in mice, and that lethal pneumonia in these animals occurred even after extensive dilution of the inoculum. In most cases of influenza in human beings, clinical pneumonia is not seen, and this paucity of pulmonary involvement has been especially notable in the epidemics that have occurred in the period intervening between the pandemics of 1918-1919 and 1957-1958. Nevertheless, it is well recognized that pneumonia is the main cause of death from influenza, and observations during the pandemic of 1918-1919 indicated that secondary or concomitant bacterial infection of the lung was almost always present in fatal cases. In fact, there were probably no more than two cases reported at that time in which the patient died of pneumonia and bacteria were not cultivated from the lung [1] and only two more in the succeeding years until 1957 [2,3]. In view of this nearly universal presence of bacteria in the lungs of patients with fatal influenzal pneumonia, it has been difficult to assess the relative importance of viral and bacterial factors in causing death.

Recent studies during the pandemic of Asian influenza have clarified this problem considerably, and now the evidence indicates that significant numbers of patients died of viral pneumonia per se. In many cases influenza virus was isolated from pulmonary tissue and bacteria were not demonstrated in the lungs. Microscopic lesions were those to be expected of influenza viral

pneumonia. The presence of virus and the absence of bacteria have been reported in approximately fifty-seven cases by seven groups of investigators [4–13] so that the validity of such observations appears to be well established.

There are at least two reasons for the contrast between the findings of the pandemics of 1918-1919 and 1957-1958. In the intervening thirtynine years a mass of knowledge about influenza virus has been obtained, and methods for its detection in pulmonary tissue are remarkably efficient. Also, during this time antibacterial therapy has had an extensive career, and it seems likely that many of the negative examinations for bacteria in lungs of patients with fatal influenzal pneumonia were the result of antibacterial treatment. Since experimental [14,15] and clinical evidence [16] do not indicate that antimicrobial therapy has any effect on the influenza viral component of pulmonary infection, isolation of influenza virus and negative cultures for bacteria suggest that the patients died of viral infection. If bacteria had been present but were eradicated or inhibited by antimicrobial therapy, the resulting therapeutic effect was not sufficient to save the patient and gives further indication of the significance of viral infection.

In many of the reports, deaths from influenza viral pneumonia occurred in patients with antecedent disease especially of the cardio-vascular system; patients with mitral stenosis appeared to be especially vulnerable [4–8].

Furthermore, in patients with mitral stenosis who eventually recovered the clinical manifestations of influenza were often severe, with dyspnea, cyanosis and hemoptysis [17,18]. Speculations concerning the reasons for such an association have included an increased demand on the heart from fever [18], decreased pulmonary compliance [7], pulmonary hypertension [8,18] and pulmonary edema [17]. That myocarditis may be a factor in some cases is suggested also by demonstration of inflammatory lesions in the myocardium [5,10–12,19].

In addition to these hypotheses concerning the relation of cardiovascular disease to influenza viral pneumonia there is another possible factor that I have not seen discussed in this connection, although it has experimental basis in controlled studies with animals. It has been established that the intranasal or intrabronchial instillation of fluids into the lungs of mice with sublethal influenza viral pneumonia converts this infection into a lethal one [14,20]. Physiological saline solution, broth, distilled water and normal serum are examples of effective fluids. Administration of fluids by aerosol does not have this effect although aerosols containing influenza virus are remarkably efficient for induction of primary influenzal pneumonia in mice. Instillation of influenzal antiserum is not followed by lethal viral pneumonia, evidently because the virus is neutralized by antibody.

Quantitative studies of the viral content of mouse lung in sublethal infection have shown that the lung contains many thousand times as much virus as is needed to produce death of mice [20]. Therefore, it may be that the effect of instilling fluid can be explained in the following manner. In the original exposure to influenza virus, only a small portion of the virussusceptible cells come into contact with the virus and are infected. Release of virus from such cells is not followed by significant spread of virus to other susceptible cells because their surfaces are in contact with the air of bronchioles or alveoli. On the other hand, when fluid is instilled into the air passages, virus from the surfaces of the infected cells is transported to other susceptible cells and a fatal pneumonia results. Transport of viral particles to susceptible cells would thus be closely analogous to the spread of bacteria such as pneumococci to previously uninvolved portions or lobes of lung. Studies of the spread of pneumococci in the lungs of experimental animals have shown very

well that the bacteria are transported by means of edema fluid.*

If edema fluid spreads influenza virus in the human lung as instilled fluid appears to do in the mouse, it seems likely that pulmonary edema from cardiovascular or other causes may serve to intensify the severity of the viral infection and may be an important factor in fatal outcome under such conditions. However, regardless of the validity of these speculations, it seems clear that pure influenza viral pneumonia occurs and there is a strong suggestion of an association between fatal influenza viral pneumonia and cardiovascular disease. Whether improvements in supportive therapy such as technics for administration of oxygen [10] will have any significant effect in saving such patients is difficult to say. It does seem, however, that optimal use of influenza vaccine should be an integral part of the long-term care of patients with cardiovascular disease.

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REFERENCES

- GOODPASTURE, E. W. The significance of certain pulmonary lesions in relation to the etiology of influenza. Am. J. Med. Sc., 158: 863, 1919.
- FINLAND, M., BARNES, M. W. and SAMPER, B. A. Influenza virus isolations and serological studies made in Boston during the winter of 1943

 –44. J. Clin. Invest., 24: 192, 1945.
- PARKER, F., JR., JOLLIFFE, L. S., BARNES, M. W. and FINLAND, M. Pathologic findings in the lungs of five cases from which influenza virus was isolated. Am. J. Path., 22: 797, 1946.
- Hers, J. F. P., Goslings, N. R. O., Masurel, N. and Mulder, J. Death from Asiatic influenza in the Netherlands. *Lancet*, 2: 1164, 1957.
- Giles, C. and Shuttleworth, E. M. Post-mortem findings in 46 influenza deaths. *Lancet*, 2: 1224, 1957.
- Hers, J. F. P., Masurel, N. and Mulder, J. Bacteriology and histopathology of the respiratory tract and lungs in fatal Asian influenza. *Lancet*, 2: 1141, 1958.
- ROCK, J. A., BRAUDE, A. I. and MORAN, F. J. Asian influenza and mitral stenosis. Report of a case with autopsy. J. A. M. A., 166: 1467, 1958.
- autopsy. J. A. M. A., 166: 1467, 1958.

 8. LOURIA, D. B., BLUMENFLED, H. L., ELLIS, J. F., KILBOURNE, E. D. and ROGERS, D. E. Studies on influenza in the pandemic of 1957–1958. II.
- * Papers describing this phenomenon are listed in reference 21.

- Pulmonary complications of influenza. J. Clin. Invest., 38: 213, 1959.
- KILBOURNE, E. D. Studies on influenza in the pandemic of 1957–1958. III. Isolation of influenza A (Asian strain) viruses from influenza patients with pulmonary complications. Details of virus isolation and characterization of isolates, with quantitative comparison of isolation methods. J. Clin. Invest., 38: 266, 1959.
- MARTIN, C. M., KUNIN, C. M., GOTTLIEB, L. S., BARNES, M. W., LIU, C. and FINLAND, M. Asian influenza A in Boston, 1957–1958. I. Observations on thirty-two influenza-associated fatal cases. Arch. Int. Med., 103: 515–1959.
- Oseasohn, R., Adelson, L. and Kaji, M. Clinicopathologic study of thirty-three fatal cases of Asian influenza. New England J. Med., 260: 509, 1959.
- Kaji, M., Oseasohn, R. and Jordan, W. S., Jr. Isolation of Asian virus from extrapulmonary tissues in fatal human influenza. Proc. Soc. Exp. Biol. & Med., 100: 272, 1959.
- PETERSDORF, R. G., FUSCO, J. J., HARTER, D. H. and ALBRINK, W. S. Pulmonary infections complicating Asian influenza. Arch. Int. Med., 103: 262, 1959.
- HARFORD, C. G., SMITH, M. R. and WOOD, W. B., JR. Sulfonamide chemotherapy of combined infection with influenza virus and bacteria. *J. Exp. Med.*, 83: 505, 1946.

- EATON, M. D. Chemotherapy of virus and rickettsial infections. Ann. Rev. Microbiol., 4: 223, 1950.
- THALMANN, W. G., KEMPE, C. H., WORRALL, J. A. and MEIKLEJOHN, G. Aureomycin in the treatment of influenza. A controlled study. J. A. M. A., 144: 1156, 1960.
- NEWCOMBE, C. P., NIXON, P. G. F. and THOMPSON, H. Influenzal pneumonia in mitral stenosis. *Acta Med. Scandinav.*, 162: 441, 1958.
- WALSH, J., BURCH, G. E., WHITE, A., MOGABGAB, W. and DIETLEIN, L. A study of the effects of type A (Asian strain) influenza on the cardiovascular system of man. *Ann. Int. Med.*, 49: 502, 1958.
- FINLAND, M., PARKER, F., JR., BARNES, M. W. and JOLIFFE, L. S. Acute myocarditis in influenza A infections. Two cases of non-bacterial myocarditis with isolation of virus from the lungs. Am. J. Med. Sc., 209: 455, 1945.
- TAYLOR, R. M. Experimental infection with influenza A virus in mice. The increase in intrapulmonary virus after inoculation and the influence of various factors thereon. J. Exp. Med., 73: 43, 1941.
- 21. Harford, C. G. and Hara, M. Pulmonary edema in influenzal pneumonia of the mouse and the relation of fluid in the lung to the inception of pneumococcal pneumonia. J. Exp. Med., 91: 245, 1950.

The Mechanism of Cardiovascular Action of Nitroglycerine*

An Example of Integrated Response During the Unsteady State

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N the centenary of the discovery of their vascular effects [1] nitrates are, after digitalis, the most commonly prescribed drugs which act primarily on the circulation. Nevertheless, cardiac output, coronary blood flow, ventricular work and myocardial efficiency have been reported to be increased, decreased or unchanged by nitroglycerine [2-16]. Since investigators cannot agree as to what nitroglycerine does, the mechanism by which it ameliorates angina or, paradoxically, precipitates or aggravates infarction remains unknown. The available evidence supports two mutually compatible theories, both of which require knowledge of the oxygen supply of the heart in relation to its work load for experimental verification. Classic theory attributes the action of nitroglycerine to coronary vasodilatation and increased coronary blood flow [3-7,9,10,13,14]. Others believe coronary flow to be diminished or unchanged, and attribute relief of ischemia to reduced cardiac work and oxygen requirement [2,8,12,15,16].

The root of the problem and of the controversy lies in the fact that nitroglycerine produces a rapidly fluctuating unsteady state. Under such conditions it is hardly meaningful to compare a variable with its control value unless the time required for its measurement is negligible, and a particular point in the sequence of events is specified. Unfortunately, most previous studies

in dogs, and all those performed in man, are based on observations of mean flow over an appreciable time, using methods which depend for their validity upon the existence of a steady state [17,18].

In the following experiments, technics appropriate to the study of circulatory change are used to re-examine the hemodynamic effects of nitroglycerine, and to analyse the determinants of nitroglycerine responses.

METHODS

Simultaneous measurements of phasic pressure and flow in the ascending aorta were made in ten dogs. In seven, a hydrometric pendulum meter and light pentobarbital anesthesia were used; in three, light chloralose anesthesia and a square wave electromagnetic flow meter were employed. No qualitative difference in response to nitroglycerine was apparent between these preparations. The pendulum meter consisted of a "T" cannula into which a specially tapered blade extended a distance equal to the cannula radius. Deflection of the blade in proportion to linear velocity was detected as resistance change. A system of slide and ways, operated from the outside of the closed chest, removed the sensing element from the stream for registration of zero flow. The meter was inserted into the ascending aorta during total venous occlusion, and was calibrated outside the body with sinusoidal velocities generated with a piston pump. The fidelity of pendulum type meters has been recently confirmed by Taylor [19].

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The electromagnetic meter* was manufactured by Kiger-Dennard Associates, Winston-Salem, N.C., model No. 102 [20]. The instrument permits registration of flow in an unopened vessel, and obviates the need for venous occlusion. Zero was taken to be the end diastolic point on the flow pulse, an approximation previously used by others [21].

Both meters fix aortic cross section at the point of measurement; stroke volume could therefore be obtained by planimetric integration and stroke acceleration by electrical differentiation.

Aortic pressure was measured with a Statham P23D transducer either through lead tubing attached to the flow cannula, or through a No. 8 x-ray catheter. Left atrial pressure was measured via a small pulmonary vein.

Data for work calculations were registered on paper moving at 75 or 100 mm. per second. In some experiments a second polygraph driven 0.25 mm. per second permitted visualization of the time course in relation to calculated data. Representative beats before and at approximately ten second intervals after the administration of nitroglycerine were analyzed as follows: pressure, volume, flow rate and linear velocity were read directly from the record at 5 or 10 msec. intervals throughout systole. Corresponding values of pressure and flow rate were multiplied, and the products plotted as a function of systolic time. The resulting bell-shaped curve describes power output, while the planimetrically determined area beneath it represents left ventricular pressure-volume work less that small amount expended in the coronary circuit. As a check, corresponding values for pressure and fractional stroke volume were multiplied and plotted cumulatively as a function of systolic time. The resulting "S" shaped curve describes pressure-volume work per stroke. There was no consistent difference between the results of the two types of work calculations. Kinetic work was similarly computed from fractional stroke masses and linear velocities, and added to pressure-volume work to obtain total external work per stroke.

In five preparations oxygen tension in the left ventricular myocardium was recorded as a reference variable. Continuously polarized, bare platinum microcathodes 0.5 mm. in diameter and 2 mm. long were connected in a bridge circuit with a standard calomel half-cell. Current in the circuit is proportional to the rate of reduction of oxygen at the platinum surface. The system is so arranged that rate of reduction is limited by rate of diffusion of oxygen to the cathode, which in turn is chiefly determined by oxygen tension in the surrounding tissue [22]. The baseline is stable and is not significantly affected by cardiac motion [23]. Cathodes inserted in tissue cannot be

* We gratefully acknowledge, the assistance of Dr. William M. Chardack, Chief, Surgical Service, Buffalo Veterans Administration Hospital, in whose laboratory these three experiments were performed.

calibrated in units of oxygen tension. The results are therefore expressed in microamperes, a satisfactory compromise when an index of change rather than an absolute value is desired [24].

Determinants of the cardiac output response to nitroglycerine were further studied in twenty-four intact, three spinal and two carotid denervated vagotomized animals. Since flow was measured at the level of the second intercostal space, these preparations could not be used to study cardiac work. Inferior vena caval flow was measured in two additional dogs.

Tight chest closure and complete reduction of pneumothorax were found to be absolutely essential. To facilitate closure, broad chested dogs were selected, atelectasis carefully reduced, and chest catheters installed bilaterally. Positive airway pressure maintained the lungs fully expanded against the chest wall as the last sutures were placed. To insure an airtight seal, the incision was then covered with a heavy layer of collodion or vaseline gauze. Unless otherwise indicated, experiments were conducted during spontaneous respiration. Normal body temperature was maintained, and estimated blood loss replaced with warm, heparinized dog blood.

In man, sublingually administered nitroglycerine raises the blood nitrate level to half maximal concentration over sixty seconds or less [25]. To simulate the sublingual route nitroglycerine was administered to animals slowly intravenously in 5 to 10 cc. of warm saline solution. Sufficient time was allowed between experimental maneuvers to re-establish stable baselines.

Correlative observations were made in seven healthy young human adults using both an ultra low-frequency acceleration ballistocardiograph and a high-frequency vertical instrument. The underlying ballistic theory as well as the relationship of the record to blood flow have been discussed elsewhere [26,27]. Although high frequency records are less desirable than ultra low-frequency ones, the relationship between them has now been thoroughly defined [27].

Change in the distribution of blood volume, and the balance between venous return and cardiac output, were studied in eight human subjects with an isometric, recording teeterboard. The theory underlying the method has been discussed by Fenn and coworkers [28]. In brief, if a subject lies critically balanced, displacement of his center of gravity is a measure of change in distribution of blood volume, providing the rest position of the diaphragm remains unaltered. Acute shifts take place primarily between the thoracic and extremity reservoirs; the splanchnic bed seems relatively less important in man [29]. Displacement of the center of gravity was measured isometrically with a piston-type strain gauge (Statham Instruments Inc., model G1-8-35), and thoracic and abdominal pneumographs were used to monitor the rest position of the diaphragm. Either leg volume or isolated venous segment pressure was simultaneously

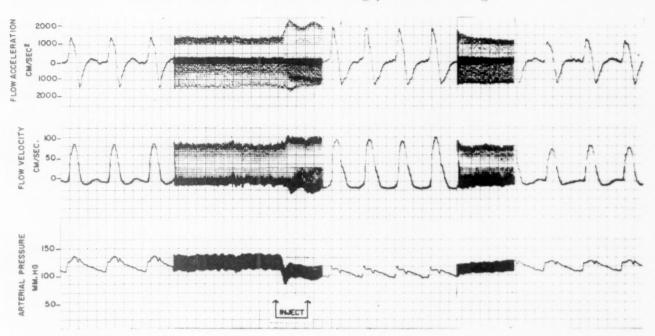


Fig. 1. Initial changes in pressure and flow following the administration of 0.3 mg, nitroglycerine intravenously to a lightly anesthetized, spontaneously breathing dog.

recorded for reference. It should be emphasized that absolute reservoir volumes were unknown; only change in distribution was measured.

RESULTS

Arterial and Venous Pressures. In anesthetized dogs, the administration of nitroglycerine regularly decreased diastolic pressure despite a simultaneous rise in cardiac output. After

thirty to forty-five seconds the diastolic pressure stabilized, then recovered during the next three to fifteen minutes despite reduction in cardiac output below its control level. (Figs. 1 and 2, Table 1.) Traube-Hering waves, if initially present, disappeared immediately after the administration of nitroglycerine and often reappeared at lower frequency but greater amplitude three to five minutes later. As the

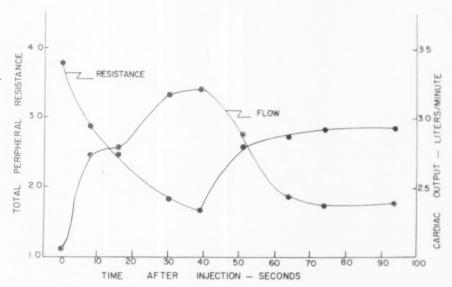


Fig. 2. Change in peripheral vascular resistance and left ventricular output following the administration of nitroglycerine. The abrupt rise in resistance forty seconds after nitroglycerine reflects an increase in aortic pressure as well as a fall in cardiac output.

diastolic pressure fell the amplitude of the anacrotic acceleration transient increased, duration of the systolic plateau decreased, and the dicrotic notch assumed a lower position. The reverse changes were associated with recovery.

Indirect arterial pressure measurements were made in human subjects in conjunction with teeterboard and ballistocardiographic observations. With the onset of flush and/or throb, the diastolic pressure fell slightly, and pulse pressure decreased. Similar findings have been reported by others [4,5]. The difference in pulse pressure response between dog and man probably represents failure of the initial acceleration transient to produce an arterial sound [30].

In seven subjects pressure was measured in an occluded segment of forearm vein [31]. As symptoms appeared and the teeterboard record indicated a shift of center of gravity of the body, footward pressure in the segment rose 5 to 25 mm. Hg, remained high for one to three minutes, then fell well below control levels. The initial rise represents reflex venoconstriction, while the secondary fall is a direct effect of nitroglycerine in the surrounding tissues on the smooth muscle of the venous segment. Venoconstriction aroused by psychic stimuli uniformly exceeded that which could be produced reflexly with either nitroglycerine or the Valsalva maneuver.

Right and left atrial pressures fell abruptly after the administration of nitroglycerine and recovered slowly over the following ten to twenty minutes. Similar changes have been reported in normal man, and in patients with arteriosclerotic heart disease [13,15,16].

Heart Rate, Blood Flow and Resistance. Stroke volume, blood velocity and blood acceleration increased in every intact animal as the diastolic pressure fell. The peak increase in cardiac output ranged between 10 and 55 per cent of the control, and usually coincided with the nadir of diastolic pressure. Flow remained elevated for as long as three and a half minutes, then fell 10 to 20 per cent below the control level for two to five minutes. Blood acceleration increased proportionately more than stroke volume and linear velocity, although all three modalities always varied in the same direction. (Fig. 1.) A second abrupt increase in flow unaccompanied by pressure change often occurred three to five minutes after the administration of the drug in lightly anesthetized animals.

Figure 2 demonstrates the initial changes in cardiac output and resistance in a typical experi-

(A microamperes) A Myocardial SPONTANEOUSLY 0.3 MG. NITROGLYCERINE TO 01 223 223 223 221 122 221 252 458 458 458 458 458 Resistance ("R" units) OF FOLLOWING ADMINISTRATION 0411401440191 Whele Cycle Mean Pressure (mm. Hg) 42. 27. 28. 28. 28. 29. 29. 33. 33. 33. 148 136 137 137 137 137 137 137 CHANGES 2201444-212-4 HEMODYNAMIC 162 162 163 169 169 170 171 171 164 174 174 174 0 110 115 120 30 40 60 80 80 80 80 80

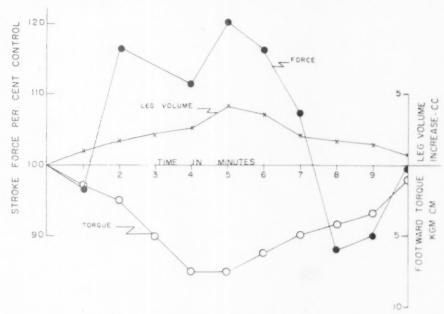


Fig. 3. Effect of nitroglycerine on blood volume distribution and the ultra low-frequency ballistocardiogram in a normal human subject. Abscissa indicates time after the administration of nitroglycerine.

ment. Resistance is computed in "R" units which are defined as the ratio of whole cycle mean pressure divided by stroke volume X heart frequency/60. The smoothly falling resistance curve exhibits a sharp inflection at forty seconds, due to both a rise in diastolic pressure and a fall in cardiac output. Such an abrupt change could hardly be attributed to destruction of nitrate, and indicates compensatory vasoconstriction. The subsequent time course, therefore, represents the algebraic sum of direct nitroglycerine effects and reflexly mediated compensatory responses.

In two dogs the administration of nitroglycerine decreased steady flow and increased pulsatile flow in the thoracic portion of the inferior vena cava. Tachypnea accompanying the initial hypotension exaggerated the slow fluctuations of venous flow attributable to the respiratory "suction pump." The net effect was a decrease in venous return, the magnitude of which varied widely with experimental conditions.

Ballistocardiographic Findings. In view of the importance of phasic flow measurements in dogs for interpretation of the effects of nitroglycerine at least qualitative observations in unanesthetized man seemed essential. To this end, an ultra low-frequency ballistocardiograph was employed as a "bloodless flowmeter." The terms "ballistic amplitude" or "force" as used in this

report denote peak to peak amplitude of the major systolic headward deflection (I-J). To clarify the relationship between flow and the ballistocardiogram under the influence of nitroglycerine the two variables were recorded simultaneously in six dogs. Since they uniformly exhibited parallel change, we have interpreted human ballistocardiograms as indicating directional change in cardiac output. The peak rise in ballistic amplitude in man ranged between 10 and 35 per cent of the control, and some increase persisted for up to eight minutes. The duration of the ballistic response was therefore more than twice the most prolonged flow increase in dogs. It seems reasonable to attribute this time difference, at least in part, to orthostatic factors, for in human subjects tested on the vertical ballistocardiograph force remained high for only one and a half to four minutes.

Distribution of Blood Volume. Figure 3 demonstrates a typical teeterboard and plethysmographic response in relation to the ultra low-frequency ballistocardiogram of the same person recorded at a subsequent time. Note that the ballistocardiogram indicates an increase in cardiac output as the center of gravity of the body is displaced towards the legs. Control distribution of blood volume is restored as the ballistic amplitude falls. These observations fit well with direct measurements of blood flow in the thoracic inferior vena cava of dogs, and

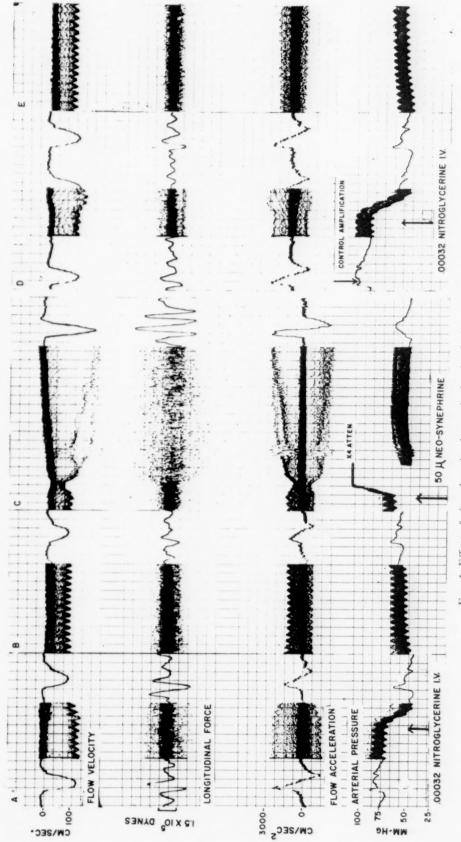


Fig. 4. Effect of nitroglycerine in a spinal animal; discussion in text.

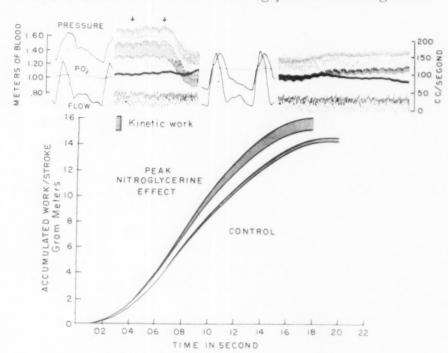


Fig. 5. Note that both myocardial oxygen tension and stroke work increase in response to nitroglycerine despite reduced diastolic pressure and cycle length.

indicate that nitroglycerine increases left ventricular output to a greater extent than venous return to the right side of the heart. The difference between venous inflow and aortic outflow is drawn from the "buffer" blood volumes in the thoracic reservoirs.

Determinants of Hemodynamic Responses. The following experiments were undertaken to (1) determine the extent to which nitroglycerine responses may be modified by manipulating venous return, and (2) differentiate direct effects of nitroglycerine from compensatory reflex changes.

Figure 4 demonstrates the effect of nitroglycerine in a spinal animal. Ventilation was maintained with a Seeler demand valve adjusted so that positive and negative phases were of equal magnitude and duration. In panel A, control aortic flow exhibits as much fluctuation as is normally expected in the pulmonary artery, suggesting a small central venous reservoir. Nitroglycerine actually decreases cardiac output, and recovery does not occur (panel B) unless venous return is improved. In panel C, the thoracic reservoir is experimentally "primed" by intense vasoconstriction. Nitroglycerine now increases cardiac output for approximately thirty seconds (panel D), but the subsequent fall is almost as profound as in panel B. Similar brief increases in flow could be produced in the

head down position, after administration of large volumes of dextran, and during negative pressure breathing, but the response of the same animal prior to spinal transection could never be completely duplicated.

Carotid denervated, vagotomized dogs behaved much like spinal animals, indicating that the control level of vasomotor tone is less important than the capacity to adjust. It is unlikely that all afferent pathways involved in compensation for acute vasodilatation involve carotid and aortic baroreceptors and the cervical vagus [32]. Nevertheless, these would appear to be the principal and essential ones.

Using the photofluorometric technic for the estimation of circulating catecholamines we have observed that nitroglycerine rapidly raises the titer in adrenal venous blood from three- to fivefold. Similar results have been reported by Darby and co-workers [33]. Part of the abrupt resistance change shown in Figure 2 may therefore be ascribed to direct stimulation of the adrenal medulla. Since pressure in an isolated venous segment rises synchronously with the onset of arteriolar dilatation, a general increase in sympathetic tone probably augments the effects of circulating humors. The importance of orthostatic venoconstriction has recently been emphasized by many authors [31–35].

Although reflex adjustments dominate re-

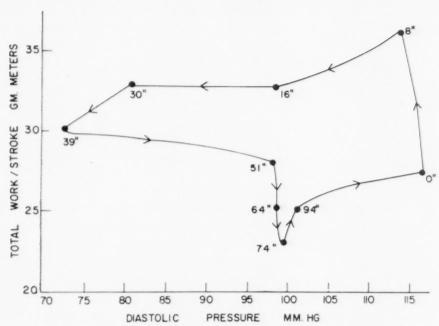


Fig. 6. The lack of correlation between changes in work and diastolic pressure is typical of acute vasodilatation when the venous return is adequate. The small numerals denote time after injection of nitroglycerine.

sponses to nitroglycerine in intact animals, the mechanical factors which "set the stage" for them also are important. Animals with a pneumothorax, or an open chest, respond to the administration of nitroglycerine much like spinal or deafferented ones. Similarly, cardiac output increased only briefly after the administration of curare, additional anesthesia, and most important, before recovery from a previous dose of nitroglycerine, or during a constant intravenous infusion of the drug. In short, the more closely the condition of the anesthetized postoperative dog resembles that of normal unanesthetized man the longer the initial flow increase and the shorter the subsequent fall.

External Useful Work of the Left Ventricle. Figure 5 displays curves of accumulated work for a control beat and a beat at peak nitroglycerine effect, together with cuttings of the oscillographic record from which the data were derived. The lower borders of the "S" shaped curves represent pressure-volume, the upper borders total work per stroke. Vertical distance across the shaded area, referred to the ordinate, represents kinetic work. Note that after the administration of nitroglycerine pressure and flow pulses rise more sharply, and their peaks correspond more closely in time. It is this change in time relationship as well as increase in stroke volume which accounts for a 5 per cent increase in pressure-

volume work, despite the reduced cycle length and profound fall in diastolic pressure. In addition, kinetic work increased 350 per cent, and total work per minute 24 per cent. Nevertheless, had work been computed in the usual manner, it would have been concluded that work had increased 4 per cent or decreased 6 per cent, depending upon the time-mean pressure selected. Clearly, kinetic work and the time relations between pressure and flow may not be neglected under conditions of acute vasodilatation.

The error in neglecting the time course of ejection is also illustrated by the differences between the effective and time-mean pressures in Table 1. Effective mean pressure is defined as that value which, when multiplied by stroke volume, gives a value for pressure-volume work equal to the true work integral. This difference increases as diastolic pressure decreases. Similarly, the use of a mean figure for linear velocity underestimates kinetic work which, properly computed, often exceeds 10 per cent of total work during vasodilatation [36]. The absolute magnitude of kinetic work also increases as diastolic pressure falls, and may account for as much as half the increase in work produced by nitroglycerine.

To emphasize the lack of correlation between pressure and work so typical of the action of nitroglycerine when venous return is adequate,

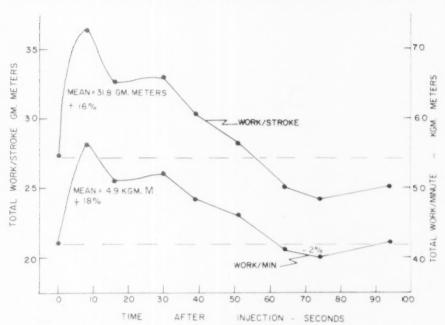


Fig. 7. Time course of work per stroke and per minute in a spontaneously breathing, lightly anesthetized dog. A second increase in work often occurs three to five minutes after injection. Despite the additional load, myocardial oxygen tension increased.

we have plotted another work response as a function of diastolic pressure. (Fig. 6.) The small numerals represent time after administration of the drug. Note that work increases to the greatest extent during the first ten seconds, and after the first minute work declines despite negligible pressure change. Conversely, work changes relatively little for thirty seconds despite first a pressure fall and then a rise, of approximately 20 mm. Hg. The same data are plotted conventionally in Figure 7, and demonstrate that under optimal conditions obtainable in anesthetized dogs work increases for one to one and a half minutes, and then falls briefly below control values. A second increase in work often occurs in lightly anesthetized animals three to five minutes after administration of the drug. On the other hand, work decreases promptly and markedly if the venous return is compromised, and remains low for a protracted period. Such a response is evident as a decrease in both pressure and flow in Figure 8, obtained in an animal with a large pneumothorax. Although peak change in work may vary plus or minus 35 per cent with respect to the control value, for reasons outlined in the section "Determinants of Hemodynamic Response" we regard an increase in work as the usual response to nitroglycerine in man.

Myocardial Oxygen Tension. The trace super-

imposed upon the upper third of the flow pulse in Figure 5 represents oxygen tension in the free wall of the left ventricle. At a time when total work per minute is increased 24 per cent with respect to the control value, myocardial oxygen tension also is significantly higher. A similar experiment is presented in Table 1. Clearly, a reduction in external useful work is not essential for an increase in oxygen availability to the heart with nitroglycerine. Conversely, reduction in load does not necessarily diminish myocardial oxygen requirement sufficiently to insure an increased quantity of available oxygen. In Figure 8 myocardial oxygen tension rises slightly during the brief initial increase in cardiac output, then drops precipitously despite obviously reduced work. Recovery of pressure and flow cannot be maintained, and all variables exhibit a secondary fall followed by gradual recovery over the next quarter hour. Antipodal effects like those contrasted in Figures 5 and 8 could be produced in each preparation by manipulating venous return. Russek and his associates have recently indicted orthostatic stress in the genesis of paradoxical angina [37]. Our data strongly support their clinical findings and therapeutic suggestions.

Although myocardial oxygen tension, cardiac output and external work usually varied in the

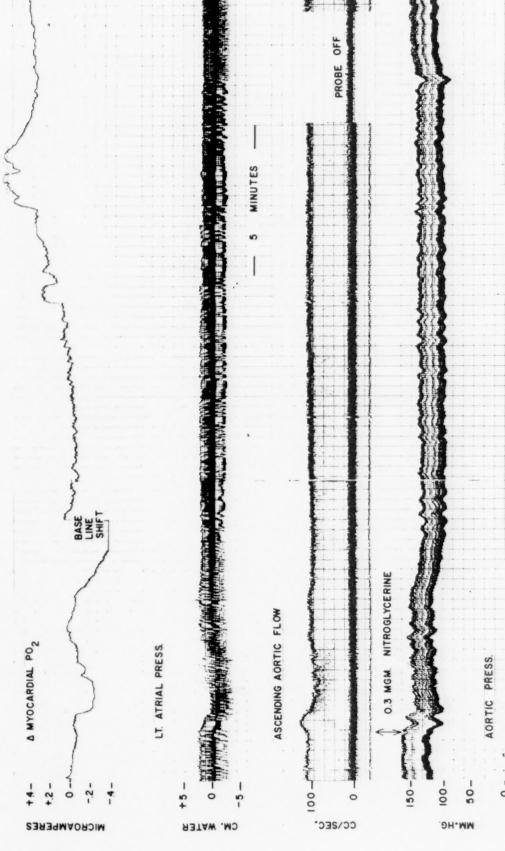


Fig. 8. Effect of nitroglycerine in the presence of pneumothorax. The pattern of response is similar for a wide variety of conditions which impede venous return. Observe that myocardial oxygen tension falls despite diminished cardiac work.

same direction, the increases in oxygen tension produced by the administration of nitroglycerine were, in general, less striking than those which occurred spontaneously or which followed the administration of epinephrine.

COMMENTS

There is now general agreement that nitrates act without prior reduction to nitrite. Glyceryl trinitrate strongly inhibits aortic ATPase activity in rats, and decreases oxygen uptake by rabbit aortic homogenates [38]. Prompt vasodilatation seems related to failure of decomposition of ATP. Vessels so poisoned may still be maximally vasoconstricted by sympathetic nerve stimulation and by epinephrine, which act elsewhere in the biochemical reactions responsible for muscle contraction [39].

Nitroglycerine certainly dilates coronary arteries and increases coronary flow in normal closed chest animals. Its capacity to dilate diseased human vessels has been questioned however by those who consider them fixed by structural change or already maximally vasodilated by the action of local metabolites [12,15,16].

Using human hearts Barbour [40] conclusively demonstrated contraction and relaxation by rings of normal, syphilytic and arteriosclerotic coronary vessels. His Case 4 indeed had extensive coronary arterial calcification. Hyaline arteriolar sclerosis is far less extensive in the heart than in other organs, and those vessels which are affected lie adjacent to others virtually free of lesions [41]. On the basis of the anatomical evidence it seems most unlikely that coronary resistance is ever completely fixed by structural change, and a fixed resistance has not been found in abnormal human hearts perfused postmortem [42]. The idea that coronary resistance is fixed as a result of maximal vasodilatation by the products of anaerobic metabolism is based on experiments by Gorlin and co-workers [15,16]. Using the nitrous oxide technic these investigators found coronary flow increased for four to twelve minutes after the administration of nitroglycerine in normal human subjects but diminished or remained unchanged in patients with coronary artery disease. Coronary resistance fell in normal subjects but appeared fixed in patients. Although these data appear to vitiate experiments conducted in normal dogs, they paradoxically invite extension of animal

research to human disease.

Gorlin's conclusions depend upon the validity of the nitrous oxide technic under the conditions of his experiments, and upon the existence of vasodilator metabolites throughout the myocardium. In respect to the latter, concentrations of lactate, pyruvate and hydrogen ion which might obtain in ischemic muscle would hardly be capable of effecting maximal vasodilatation [43], and more complex dilator metabolites have not been proved important in the regulation of coronary circulation [44,45].

Originators of the nitrous oxide method emphasized the importance of the steady state, attainment of complete equilibrium and homogeneous perfusion [46]. Sapirstein and Ogden have shown that failure to attain complete equilibrium in a non-homogeneously perfused organ may not be detected by the investigator, for arteriovenous nitrous oxide differences within the range of analytical error may represent enormous masses of poorly perfused tissue slowly coming into equilibrium [18]. Not only does the technic overestimate flow per unit weight of non-homogeneously perfused organ, but an increase in flow will actually appear as a decrease by this method. Consequently, Gorlin's data should be reinterpreted as indicating that nitroglycerine increases coronary flow in both normal subjects and patients with coronary artery disease.

In addition to coronary flow, the following determinants of myocardial oxygen tension must also be considered in evaluating drug effects: (1) the rate of O₂ consumption by non-beating muscle, (2) the distribution of coronary flow within the myocardium (3) the internal and external mechanical work, and (4) the over-all efficiency with which chemical energy is applied to load.

Metabolic rate: Krantz has shown that therapeutic concentrations of nitroglycerine are without effect on the O₂ consumption of uterine and skeletal muscle [47]. Using a modification of the Warburg technic, we have found that the oxygen uptake of cardiac muscle also is unaffected, even during the first five minutes of exposure [48]. Although epinephrine released reflexly might enhance cardiac oxygen consumption somewhat [49], the small changes one might reasonably expect would tend to decrease myocardial oxygen tension. It therefore seems highly improbable that nitroglycerine acts by altering the oxygen consumption of resting heart muscle.

Flow distribution: The effectiveness of oxygen delivery for a particular coronary flow will vary with the magnitude of anatomical and functional arteriovenous shunts [50], the pressure gradients between capillary and sinusoidal systems [51], and flow to ischemic areas through collateral channels [52]. Unfortunately, the complexity and inaccessibility of the coronary microcirculation has thus far prevented in vivo study of blood distribution among its component vessels. However, Honig and Gabel have found that nitroglycerine profoundly alters the ratio of capillary to "shunt" flow in skin and skeletal muscle [53]. A similar redistribution of blood within the myocardium is certainly possible, and would help explain the efficacy of nitroglycerine in patients with complete coronary occlusion.

Cardiac work: Chemical energy is applied to both internal and external loads. Since the former cannot be adequately studied in the intact animal, and since only change in its magnitude is relevant to the mechanism of nitroglycerine action, it will be ignored in the following discussion. External stroke work of the left ventricle (W_L) is defined as:

$$W_{\rm L} = \int_{t_0}^{t_1} \, P dV + \frac{\rho}{2g} \int_{t_0}^{t_1} v^2 dV$$

where t_{θ} is beginning of ejection, t_{1} is end of ejection, P is pressure at aortic valve, V is volume flow in ascending aorta, ρ is density of blood, v is aortic blood velocity at time t when a quantity V has been expelled and g is the gravitational constant. Let us consider each variable.

In all our experiments cardiac output changed continuously for at least a quarter hour after the administration of nitroglycerine. The magnitude of possible error in applying the Fick principle under such conditions has recently been considered by Visscher and Johnson [17]. Not only the direct Fick but also any discontinuous flow measurement will be misleading, for, as attested by the diverse results of others, the outcome will critically depend upon the time at which observations are made.

Even if these inaccuracies are accepted, cardiac work cannot be adequately represented by the simple product of time-mean pressure and flow under the conditions of nitrate vaso-dilatation. Kinetic work assumes increasing importance as flow increases, all the more so when the stroke ejection time is actually shortened. In the intact animal the error in neglecting

time and kinetic work varies directly with flow and inversely with pressure [36]. In experiments illustrated by Figures 5 and 6 for example, the usual calculation underestimated total work by 18 and 22 per cent, respectively, even though correct values for mean pressure and cardiac output were employed.

Properly computed from phasic data, work followed flow rather than pressure, and analysis of the determinants of nitroglycerine responses indicates that increased work is the usual response of the normal circulation. Nevertheless, nitroglycerine simultaneously increases oxygen tension in the ventricular wall, and in coronary venous blood [9]. Since patients with angina respond to the administration of nitroglycerine with marked increases in ballistic amplitude [10,11], it is probable that flow and external work change as in normal subjects, and that ischemia is relieved despite increased load. Conversely, if venous return is compromised in an experimental animal, nitroglycerine diminishes both work and myocardial oxygen tension. The paradoxical production of angina by nitroglycerine under conditions which embarrass venous return appears to be a clinical parallel of this response [37]. Since nitroglycerine does not regularly produce antithetical changes in work and oxygen tension in normal hearts or, on the basis of indirect evidence, in diseased ones, relief of angina seems independent of reduction in load. Clinical evidence in support of this view has been presented by others [5,10,13].

Efficiency: Chemical: Hunter and co-workers have shown that relatively low concentrations of mannitol hexanitrate inhibit electron transport phosphorylation in liver mitochondria without reducing oxygen consumption [54]. Glyceryl trinitrate was far less effective. Using mitochondria derived from cardiac muscle however, Stam and Honig observed almost complete inhibition of phosphorylation at high concentrations of nitroglycerine, as well as slight but definite inhibition with concentrations that might obtain in vivo [48]. Consequently, if nitroglycerine has any effect on cellular respiration in the intact animal it "uncouples" oxygen consumption from "high energy" phosphate bond formation and reduces chemical efficiency.

Mechanical: Mechanical efficiency, defined as the ratio of external work to oxygen consumption, can be measured in man only under steady state conditions. The reasons for this fact have already been considered. Cardiac efficiency is unchanged by nitroglycerine in the isolated but metabolically supported heart functioning at constant rate and volume [11]. When stroke volume and heart rate are free to vary, tachycardia tends to reduce efficiency [55]. On the other hand, the heart empties more completely, and therefore operates at shorter fiber lengths. Since oxygen use varies directly with fiber length, or the product of tension and time [56], reduction in residual volume should improve efficiency, particularly in dilated hearts [56,57].

We conclude that all factors bearing on efficiency may so summate as to oppose or contribute to the therapeutic action of nitroglycerine. Unfortunately, both possibilities are equally conceivable.

SUMMARY

The effects of nitroglycerine on phasic pressure, flow and myocardial oxygen tension were measured in anesthetized spontaneously breathing dogs. As aortic pressure fell, cardiac output increased from 10 to 55 per cent for half to three and a half minutes, then fell 10 to 25 per cent below control levels as the diastolic pressure recovered. Prolonged increases in flow depended upon adequate thoracic blood volume, sympathetic tonus and release of pressor amines. Under conditions most closely resembling those in man, measurements in dogs indicated a rise in left ventricular external work of as much as 25 per cent. Despite this additional load, myocardial oxygen tension increased. In contrast, when venous return was compromised both external work and myocardial oxygen tension fell. Indirect evidence is presented which suggests that qualitatively similar changes take place in patients with coronary artery disease. We conclude that nitroglycerine relieves ischemia by enhancing oxygen delivery rather than by altering load. Changes in chemical and/or mechanical efficiency may also play a role. The determinants of nitroglycerine responses are analyzed and the therapeutic implications briefly discussed.

REFERENCES

- GUTHRIE, F., 1859. In: CASH, J. T. and DUSTAN, W. R. The physiological action of the nitrites on the paraffin series, considered in connection with their chemical constitution. *Phil. Tr.*, *London*, 184: 505, 1893.
- Brunton, T. L. Lectures on the Actions of Medicines. New York, 1897. The Macmillan Co.
- 3. CAMERON, P. D. Physiological and pharmacological

studies on cardiac tonicity in mammals. Johns Hopkins Hosp. Reports, 16: 549, 1911.

 Weiss, S. and Ellis, L. B. Influence of sodium nitrite on the cardiovascular system and on renal activity in health, in arterial hypertension and in renal disease. Arch. Int. Med., 52: 105, 1933.

 WAYNE, E. J. and LAPLACE, L. B. Observations on angina of effort. Clin. Sc. 1: 103, 1933.

angina of effort. Clin. Sc., 1: 103, 1933.

6. KATZ, L. N., LINDNER, E., WEINSTEIN, W., ABRAMSON, D. I. and JOCHIM, K. Effects of various drugs on the coronary circulation of the denervated isolated heart of the dog and cat. Arch. internat. de pharmacodyn. et de thérap., 59: 399, 1938.

 ESSEX, H. E., WEGRIA, R. G. E., HERRICK, J. F. and MANN, F. C. The effect of certain drugs on the coronary blood flow of the trained dog. Am. Heart

J., 19: 554, 1940.

 Eckenhoff, J. E., Hafkenschiel, J. H. and Land-Messer, C. M. The coronary circulation in the dog. Am. J. Physiol., 148: 582, 1947.

 FOLTZ, E. L., RUBIN, A., STEIGER, W. A. and GAZES, P. C. The effects of intravenous aminophylline upon the coronary blood-oxygen exchange. *Circulation*, 2: 215, 1950.

 WEGRIA, R., NICKERSON, J. L., CASE, R. B. and HOLLAND, J. F. Effect of nitroglycerine on the cardiovascular system of normal persons. Am. J. Med., 10: 414, 1951.

 Brandt, J. L., Caccese, A. and Dock, W. Slitkymographic evidence that nitroglycerine decreases heart volume and stroke volume. Am. J. Med., 12: 650, 1952.

 ELDRIDGE, F. L., HULTGREN, H. N., STEWART, P. and PROCTOR, D. The effect of nitroglycerine upon the cardiovascular system. *Stanford M. Bull.*, 13: 273, 1955.

MULLER, O. and RORVIK, K. Haemodynamic consequences of coronary heart disease. With observations during anginal pain and on the effect of nitroglycerine. *Brit. Heart J.*, 20: 302, 1958.

14. SARNOFF, S. J., CASE, R. B. and MAGRUZ, R. Observations on the vasodilator properties of urine. 1. Comparison of the effect of human urine and nitroglycerine on coronary resistance and myocardial oxygen consumption in the isolated supported heart preparation. Circulation Res., 6: 522, 1958.

 Brachfeld, N., Bozer, J. and Gorlin, R. Action of nitroglycerine on the coronary circulation in normal and in mild cardiac subjects. *Circulation*, 19:

697, 1959.

- GORLIN, R., BRACHFELD, N., MACLEOD, C. and BOPP, P. Effect of nitroglycerine on the coronary circulation in patients with coronary artery disease or increased left ventricular work. Circulation, 19: 705, 1959.
- VISSCHER, M. B. and JOHNSON, J. A. The Fick principle: analysis of potential errors in its conventional application. J. Appl. Physiol., 5: 635, 1953.
- SAPIRSTEIN, L. A. and OGDEN, E. Theoretic limitations of the nitrous oxide method for the determination of regional blood flow. Circulation Res., 4: 245, 1956.
- Taylor, M. G. The discrepancy between steady and oscillatory flow calibration of flowmeters of the 'bristle' and 'pendulum' types. A theoretical study. Physics Med. & Biol., 2: 324, 1958.

AMERICAN JOURNAL OF MEDICINE

- Denison, A. B., Jr. and Spencer, M. P. Square-wave electromagnetic flowmeter design. Rev. Sc. Instr., 27: 707, 1956.
- FRY, D. L., NOBLE, F. W. and MALLOS, A. J. An electric device for instantaneous and continuous computation of aortic blood velocity. *Circulation Res.*, 5: 75, 1957.
- 22. Kolthoff, I. M. and Lingane, J. J. Polarography. New York, 1952. Interscience Publishers, Inc.
- SAYEN, J. J., SHELDON, W. F., HORWITZ, O., KUO, P. T., PIERCE, G., ZINSSER, H. F. and MEAD, J., JR. Studies of coronary disease in the experimental animal. II. Polarographic determinations of local oxygen availability in the dog's left ventricle during coronary occlusion and pure oxygen breathing. J. Clin. Invest., 30: 932, 1951.
- Montgomery, H. Oxygen tension of tissues in vivo. Circulation, 15: 646, 1957.
- Berry, J. W. and Roach, T. C. An evaluation of blood nitrate levels. *Circulation*, 17: 1041, 1958.
- Honig, C. R. and Tenney, S. M. The relationship between the ballistocardiogram. cardiac movement, and blood flow. Am. Heart J., 52: 167, 1956.
- Talbot, S. A. and Harrison, W. K., Jr. Dynamic comparison of current ballistocardiographic methods. III. Derivation of cardiovascular force from body motions. *Circulation*, 12: 1022, 1955.
- Fenn, W. O., Otis, A. B., Rahn, H., Chadwick, L. E. and Hegnauer, A. H. Displacement of blood from the lungs by pressure breathing. Am. J. Physiol., 151: 258, 1947.
- Tenney, S. M. Fluid volume redistribution and thoracic volume changes during recumbency. J. Appl. Physiol., 14: 129, 1959.
- Malcolm, J. E. Blood Pressure Sounds and Their Meanings. Springfield, Ill., 1957. Charles C Thomas.
- Duggan, J. J., Love, V. L. and Lyons, R. H. A study of reflex venomotor reactions in man. *Circulation*, 7: 869, 1953.
- 32. Heymans, C. and Neil, E. Reflexogenic Areas of the Cardiovascular System. Boston, 1958. Little, Brown and Co.
- DARBY, T. D., GOLDBERG, L. I., GAZES, P. C. and ARBEIT, S. R. Effects of sympathomimetic amines and nitroglycerine on the acceleration ballistocardiogram of the dog. J. Pharmacol. & Exper. Therap., 119: 248, 1957.
- Sjostrand, T. Volume and distribution of blood and their significance in regulating the circulation. *Physiol. Rev.*, 33: 202, 1953.
- SALZMAN, E. W. Reflex peripheral venoconstriction induced by carotid occlusion. *Circulation Res.*, 5: 149, 1957.
- 36. Honig, C. R. and Tenney, S. M. On computing cardiac work. *Circulation*, 16: 894, 1957.
- Russek, H. I., Urbach, K. F. and Zohman, B. L. Paradoxical action of glyceryl trinitrate (nitroglycerine) in coronary patients. J. A. M. A., 158: 1017, 1955.
- KRANTZ, J. C., JR., CARR, C. J. and KNAPP, M. J. Alkyl nitrites. 15. The effect of nitrites and nitrates on the oxygen uptake of arterial tisues. J. Pharmacol. & Exper. Therap., 102: 258, 1951.
- J. Pharmacol. & Exper. Therap., 102: 258, 1951.

 39. PILCHER, J. D. and SOLLMANN, T. Studies on the vasomotor center. 1. The effects of the nitrite

- group. J. Pharmacol. & Exper. Therap., 6: 323, 1914.
 40. Barbour. H. G. The constricting influence of
- BARBOUR, H. G. The constricting influence of adrenalin upon the human coronary arteries. J. Exper. Med., 15: 404, 1912.
- 41. GOULD, S. E. Pathology of the Heart. Springfield, Ill., 1953. Charles C Thomas.
- KOUNTZ, W. B. and SMITH, J. R. The flow of blood in the coronary arteries in pathological hearts. J. Clin. Invest., 17: 147, 1938.
- Deal, C. P., Jr. and Green, H. D. Effects of pH on blood flow and peripheral resistance in muscular and cutaneous vascular beds in the hind limb of the pentobarbitalized dog. *Circulation Res.*, 2: 148, 1954.
- Gregg, D. E. Coronary Circulation in Health and Disease. Philadelphia, 1950. Lea & Febiger.
- Jelliffe, R. W., Wolf, C. R., Berne, R. M. and Eckstein, R. W. Absence of vasoactive and cardiotropic substances in coronary sinus blood of dogs. Circulation Res., 5: 382, 1957.
- ECKENHOFF, J. E., HAFKENSCHIEL, J. H., HARMEL, M. H., GOODALE, W. T., LUBIN, M., BING, R. J. and KETY, S. S. Measurement of coronary blood flow by the nitrous oxide method. Am. J. Physiol., 152: 356, 1948.
- 47. Krantz, J. C., Jr., Carr, C. J. and Bryant, H. H. Alkyl nitrites. 14. The effect of nitrites and nitrates on arterial adenosine triphosphatase. *J. Pharmacol. & Exper. Therap.*, 102: 16, 1951.
- 48. Stam, A. and Honig, C. R. Unpublished observations.
- 49. Ellis, S. The metabolic effects of epinephrine and related amines. *Pharmacol. Rev.*, 8: 485, 1956.
- PROVENZA, D. V. and SCHERLIS, S. Coronary circulation in dog's heart. Demonstration of muscle sphincters in capillaries. *Circulation Res.*, 7: 318, 1959
- 51. Batson, O. V. and Bellet, S. The reversal of flow in the cardiac veins. *Am. Heart J.*, 6: 206, 1930.
- 52. Blumgart, H. L., Schlesinger, M. J. and Davis, D. Studies on the relation of the clinical manifestations of angina pectoris, coronary thrombosis, and myocardial infarction to the pathologic findings. *Am. Heart J.*, 19: 1, 1940.
- Honig, C. R. and Gabel, P. V. Direct effects of epinephrine and nor-epinephrine on limb vessels. Fed. Proc., 17: 73, 1958.
- HUNTER, F. E., JR., KAHANA, S. and FORD, L. Effect of inorganic and organic nitrites and nitrates on aerobic phosphorylation in liver mitochondria. Fed. Proc., 12: 221, 1953.
- MAXWELL, G. M., CASTILLO, C. A., WHITE, D. H., JR., CRUMPTION, C. W. and ROWE, G. G. Induced tachycardia: Its effect upon the coronary hemodynamics, myocardial metabolism, and cardiac efficiency of the intact dog. J. Clin. Invest., 37: 1413, 1958
- 56. SARNOFF, S. J., BRAUNWALD, W., WELCH, G. H., JR., CASE, R. B., STAINSBY, W. N. and MACRUZ, R. Hemodynamic determinants of oxygen consumption of the heart with special reference to the tension-time index. Am. J. Physiol., 192: 148, 1958.
- 57. Burch, G. E., Ray, C. T. and Cronvitch, J. A. Certain mechanical peculiarities of the human cardiac pump in normal and diseased states. *Circulation*, 5: 504, 1952.

Idiopathic Hypertrophic Subaortic Stenosis*

Clinical, Hemodynamic and Angiographic Manifestations

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In the majority of patients with obstruction to left ventricular circuit left ventricular ejection there is narrowing of the aortic valve. In others, obstruction may be caused by a discrete congenital band of fibrous tissue high in the outflow tract of the left ventricle. Occasionally, a constriction in the proximal portion of the aorta may be the site of stenosis. These lesions may be differentiated by catheterization of the left ventricle and aorta. Valvular stenosis is characterized by an increase in systolic pressure as the catheter tip is withdrawn across the valve. In subaortic stenosis the systolic pressure gradient is recorded within the left ventricle as the catheter traverses the subvalvular region, while in supravalvular aortic stenosis the pressure changes abruptly as the catheter passes the site of narrowing just above the valve. Similar lesions also occur in the right side of the heart, i.e., valvular, discrete infundibular and supravalvular pulmonic stenosis.

Recently a number of reports [1-8] have indicated that a fourth type of anatomic deformity may be responsible for obstruction to ventricular ejection. The systolic contraction of an area of diffuse muscular hypertrophy in the ventricular outflow tract may narrow this region sufficiently to result in a large systolic pressure gradient within the ventricle. Fourteen patients with idiopathic hypertrophic subaortic stenosis, a malformation characterized by systolic narrowing of the left ventricular outflow tract secondary to muscular hypertrophy of unknown etiology, have been studied at the National Heart Institute during a two-year period. A description of the clinical, hemodynamic, angiographic and operative findings in these patients forms the basis of this report.

CASE REPORTS

Case I. E. Z. (No. 00-95-04), described in detail previously with patient 2 [4], is a twenty-eight year old man who had a heart murmur for nine years, and angina pectoris and exertional dyspnea for four years. On physical examination there was evidence of left ventricular enlargement; a loud holosystolic murmur was heard at the apex and along the left sternal border. Left ventricular hypertrophy was present on both the roentgenogram and the electrocardiogram. Left heart catheterization revealed a systolic pressure gradient of 74 mm. Hg within the left ventricular outflow tract. The patient was considered to have the usual form of congenital subaortic stenosis and operation was recommended.

During the procedure, carried out with cardiopulmonary bypass and elective cardiac arrest, no discrete stenosis was evident, the aortic valve was normal, and a finger could easily be passed from the aorta to the left ventricular apex. The left ventricular wall was markedly thickened, the mitral valve leaflets were normal to palpation, but a moderate degree of mitral regurgitation was present.

Following exploration the patient's symptoms improved markedly and he resumed heavy physical work without limitation. One year after operation the physical findings and left ventricular pressures were unchanged, although the heart had enlarged further. A selective left ventricular angiocardiogram at this time revealed narrowing of the left ventricular cavity and thickening of the left ventricular wall which encroached on the outflow tract during portions of the cardiac cycle.

CASE II. C. G. (No. 01-81-10), a twenty-one year old student, was known to have had a heart murmur since the age of twelve years. Mild exertional dyspnea and anginal pain were first noted at the age of fifteen years. On physical examination the left ventricle was enlarged. A systolic thrill and ejection murmur were

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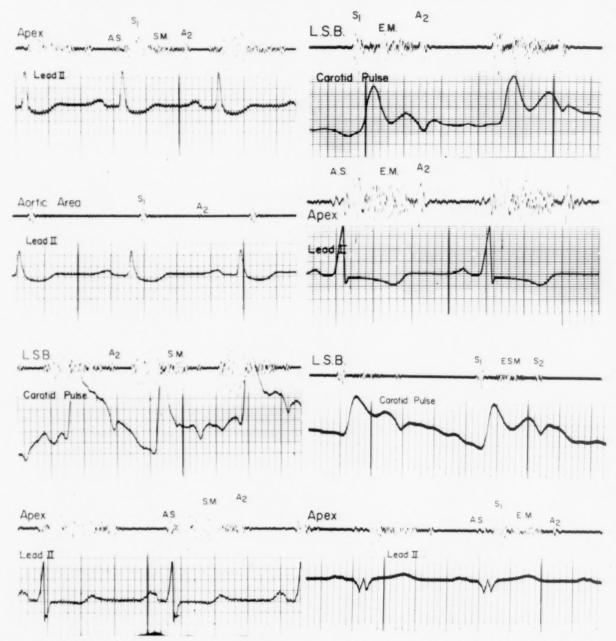


Fig. 1. Phonocardiograms. LSB = left sternal border; AS = atrial sound; S_1 = first heart sound; S_2 = second heart sound; P_2 = pulmonic closure sound; P_2 = artic closure sound; P_2 = systolic murmur; EM = ejection murmur; INSP = inspiration. Time lines equal 0.04 seconds. Top left, Case III. Top right, Case IV. Bottom left, Case VII. Bottom right, Case VIII.

present along the left sternal border and at the apex. Left atrial and ventricular enlargement were evident on both the roentgenogram and the electrocardiogram. A 72 mm. Hg systolic pressure gradient was present within the left ventricular outflow tract.

Operation for what was believed to be the usual form of congenital subaortic stenosis was carried out with cardiopulmonary bypass and elective cardiac arrest. Again, as in the first patient (Case 1) no obstruction at the aortic valve or within the left ventricle could be detected. Left ventricular thickening and

mild mitral regurgitation with normal mitral valve leaflets were present.

A selective left ventricular angiocardiogram was carried out three months after operation. There was mitral regurgitation and the left ventricular outflow tract narrowed markedly in systole, but relaxed during diastole. An intrinsic mass, presumably hypertrophied cardiac muscle, appeared almost to divide the left ventricle into two separate chambers. In the lateral films the left ventricular outflow tract was shaped like an inverted cone, with its base at the aortic valve

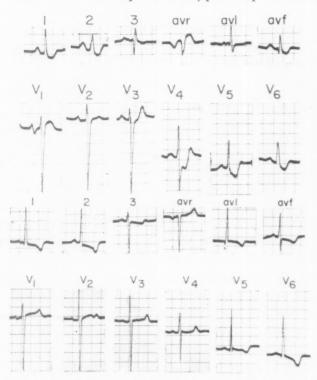


Fig. 2. Electrocardiograms. Top, Case III. Bottom, Case IV.

and the obstruction appeared to be at the apex of the cone [4].

Case III. M. H. (No. 02-49-10), a thirty-nine year old housewife, was admitted to the National Heart Institute in June 1959. A heart murmur had been discovered at the age of twenty-six years during the course of her second pregnancy, but there was no history of rheumatic fever. Exertional dyspnea and fatigability were first noted four years prior to admission and for two years she had been in overt congestive heart failure, with edema of the ankles, abdominal swelling and frequent episodes of paroxysmal nocturnal dyspnea requiring digitalis and diuretics. In addition, she also complained of frequent episodes of pain in the left side of the chest, precipitated by exertion and lasting one to two hours.

On physical examination the blood pressure was 110/70 mm. Hg, the pulse was regular and normal to palpation. A prominent left ventricular lift was palpable. The first heart sound was normal, the second sound showed paradoxical splitting and an atrial sound was present at the apex. A grade 4/6 holosystolic murmur was most prominent at the apex and was widely transmitted over the precordium and the back, but was almost inaudible in the aortic area and along the carotid vessels. (Fig. 1.) The liver edge was palpable 2 fingers below the right costal margin and was tender.

The electrocardiogram (Fig. 2) revealed digitalis effect as well as left atrial and left ventricular enlargement. Both of these chambers were also moderately

enlarged on roentgenographic examination. (Fig. 3.) Transbronchial left heart catheterization demonstrated that the mean left atrial pressure was 20 mm. Hg; the left atrial pulse contour was not suggestive of mitral regurgitation. A peak systolic pressure gradient of 75 mm. Hg between the left ventricle and brachial artery and an 8 mm. Hg mean diastolic pressure gradient between the left atrium and left ventricle were present. The effective "aortic" orifice size was 0.50 sq. cm. [9]. Both the clinical picture and the rapid upstroke of the brachial artery pressure pulse (Fig. 4, top) were considered atypical for valvular aortic stenosis. Accordingly, a retrograde arterial left ventricular catheterization was carried out and this revealed an 80 mm. Hg systolic gradient within the outflow tract of the left ventricle; the end diastolic left ventricular pressure was elevated to 25 mm. Hg, largely as a result of atrial contraction. Proximal to the obstruction the left ventricular pressure pulse exhibited pulsus alternans and, in addition, there was a notch on the ascending limb at a level which corresponded to the peak systolic pressure distal to the obstruction. (Fig. 5.) The peak pressure distal to the obstruction occurred early in ventricular systole, and fell off later in systole. The left ventricular angiocardiogram (Fig. 6) revealed marked generalized thickening of the left ventricular wall, and a filling defect extending into the left ventricular cavity from the inferior aspect of the ventricular wall during systole; the left ventricular cavity enlarged during diastole and considerable regurgitation of contrast material into a greatly enlarged left atrium was noted.

Although the diagnosis of idiopathic hypertrophic subaortic stenosis was strongly suspected, the possibility of a discrete obstructing lesion could not be excluded with certainty at the time this patient was studied. In view of her progressively severe incapacitation, exploration was elected. At operation there was no poststenotic dilatation of the aorta and only a faint systolic thrill was palpable in it. A mild regurgitant jet was felt in the left atrium, but careful palpation of the mitral leaflets revealed that they were free and mobile and there was no palpable calcification or thickening. With the aid of extracorporeal circulation and cardiopulmonary bypass the ascending aorta was incised. Myocardial contraction was not interrupted since the left coronary artery was perfused with oxygenated blood. Inspection of the aortic valve revealed it to be entirely normal. A finger could be passed to the left ventricular apex without difficulty. No localized obstruction of any type could be felt. A muscular ridge, 2.5 cm. in width, with its superior margin approximately 2.5 cm. below the aortic valve, was palpable. This ridge contracted around the surgeon's finger with each systole and relaxed during diastole, offering no obstruction during the latter phase. It was decided that no attempt should be made to resect ventricular

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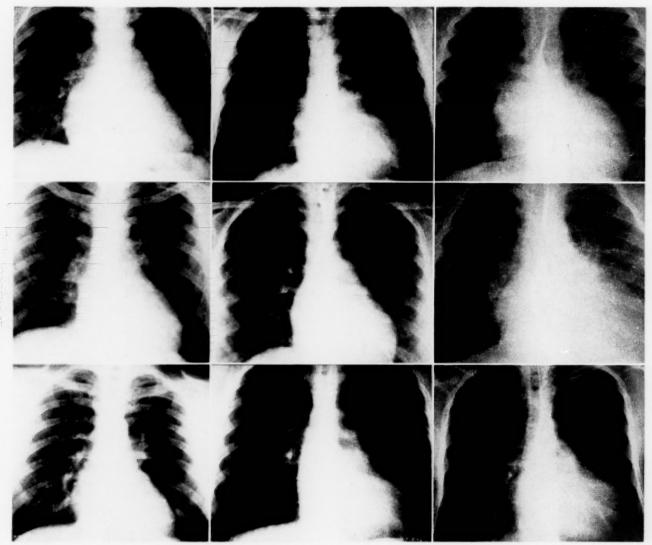


Fig. 3. Anteroposterior roentgenograms, reading from top to bottom. Left, Cases III, IV and V. Middle, Cases VI, VII and VIII. Right, Cases IX, X and XI.

muscle and the aortotomy was closed. The patient's postoperative course was complicated by prolonged hypotension, but at the time of discharge her condition had returned to that present on admission.

CASE IV. D. E. (No. 01-81-23), a thirty-seven year old white Air Force pilot, was admitted to the National Heart Institute for study in June 1959. There was no family history of heart disease and he had not had rheumatic fever. The patient had been in excellent health and had repeatedly passed the strict physical examinations required of pilots. In 1957, during an annual examination, a grade 3/6 apical systolic murmur was discovered for the first time, but there was no evidence of cardiac enlargement on the roentgenogram. He was seen by two experienced cardiovascular consultants, one of whom thought that he had rheumatic mitral regurgitation while the other indicated that the physical findings

were more characteristic of a ventricular septal defect. When examined one year later there were no changes in the findings on clinical examination. He was referred for study when the next annual examination revealed clear evidence of increasing left ventricular hypertrophy, although he had remained entirely asymptomatic.

On physical examination the blood pressure was 130/80 mm. Hg and his peripheral pulses were normal and regular. There was moderate cardiac enlargement with a prominent left ventricular thrust. The heart sounds were of good quality and a protodiastolic gallop was heard at the apex. A grade 3/6 high-pitched ejection murmur was present over the entire precordium, most prominently at the apex but also well heard along the left sternal border and in the axilla. The phonocardiogram (Fig. 1) revealed the presence of an atrial sound and correlation with the carotid pulse indicated that the delayed second heart

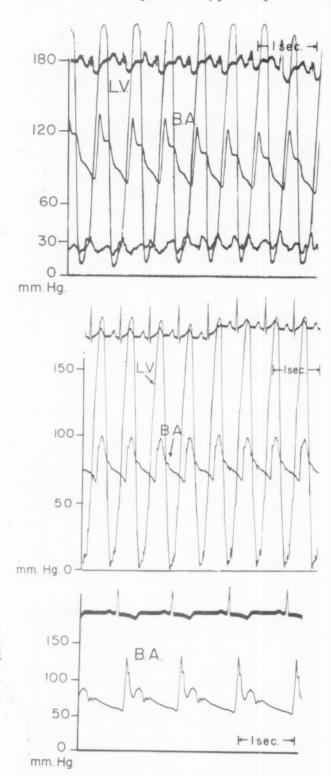


Fig. 4. Top, Case III. Simultaneous left atrial (LA), left ventricular (LV), and brachial artery (BA) pressure pulses. The gradients between the left atrium and ventricle, and between the left ventricle and brachial artery are evident. *Middle*, Case vi. Simultaneous left ventricular and brachial artery pressure tracings. *Bottom*, Case IV. Brachial artery pressure pulse.

sound was due to aortic valve closure. The pulmonic component of the second sound could not be identified. It was either superimposed on the aortic component or masked by the systolic murmur. The indirect carotid pulse tracing revealed an abrupt ascending limb, interrupted in early systole and followed by a second smaller positive deflection. The electrocardiogram (Fig. 2) indicated left ventricular hypertrophy and the roentgenogram (Fig. 3) revealed enlargement of the left ventricle without poststenotic dilatation of the aorta or valvular calcification.

The cardiac output was normal at rest (2.71 L., minute/sq. m.), but failed to increase normally with exercise. The brachial artery pressure pulse (Fig. 4. bottom) showed an abrupt ascending limb, unlike that usually seen in aortic stenosis. Transbronchial left heart catheterization revealed a 110 mm. Hg peak systolic pressure gradient between the cavity and the outflow tract of the left ventricle; the calculated effective orifice size was 0.37 sq. cm. A retrograde left ventricular catheterization was performed and the systolic gradient across the outflow tract of the left ventricle was again demonstrated. (Fig. 5.) The peak pressures in the left ventricular outflow tract and central aorta were developed in early systole. The left ventricular end diastolic pressure was elevated to 26 mm. Hg by atrial systole and the notch on the upstroke of the left ventricular pressure pulse noted in Case III was present. (Fig. 5.) The left ventricular angiocardiogram (Fig. 7) revealed indentation of the inferior and medial aspects of the left ventricle by a large muscle mass which resulted in marked narrowing of the outflow tract; when the outflow tract was narrowed it demonstrated the inverted cone shape in lateral views and mild mitral regurgitation was also present. The films showed the catheter to be within the myocardium, and extravasation of contrast material into the myocardium and pericardial cavity was apparent. When the arterial pressure fell several hours after the procedure a thoracotomy was performed and approximately 200 cc. of blood were removed from the pericardial cavity. Exertional dyspnea and orthopnea were present during the postoperative period but these have gradually regressed.

Case v. W. D. (No. 02-48-16), a thirty-seven year old man, was admitted to the Clinical Center in June 1959 for diagnosis. His family history was non-contributory for heart disease. There was no history suggestive of rheumatic fever. At the age of twenty years he had been inducted into the military service. At the age of thirty-five years he was first informed of the presence of a heart murmur. However, he remained in excellent health and had no complaints referable to the cardiovascular system until early 1959 when he experienced two episodes of syncope not associated with convulsions. He had never experienced dyspnea, orthopnea or angina.

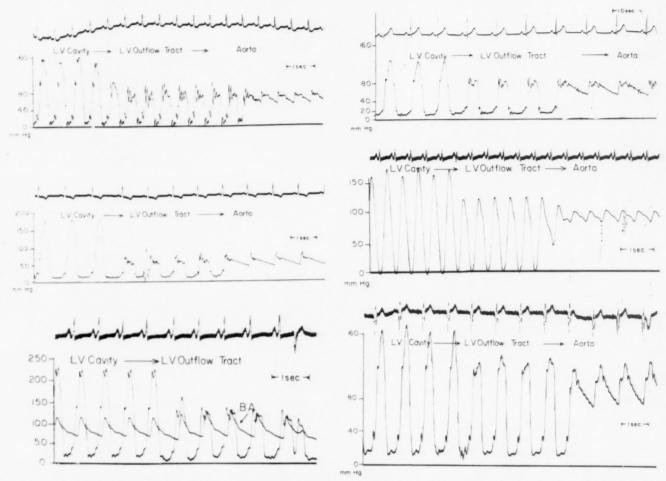


Fig. 5. Continuous pressure recording as catheter was withdrawn from the main left ventricular (LV) cavity, through the outflow tract and across the aortic valve into the aorta. Reading from top to bottom. *Left*, Cases III, IV and V. *Right*, Cases VI, VII and VIII.

On physical examination the blood pressure was 140/80 mm. Hg and the peripheral pulses were normal. The heart was slightly enlarged to the left; a left ventricular lift and a systolic thrill were present at the apex and along the left sternal border. The heart sounds were normal, there was normal splitting of the second heart sound at the base. A grade 4/6 pansystolic murmur was heard at the apex, in the axilla and along the left sternal border. The murmur was not transmitted to the neck. A protodiastolic sound was heard at the apex. The remainder of the physical examination including the neurological examination was within normal limits. The electrocardiogram (Fig. 8) showed anomalous atrioventricular excitation with a short P-R interval, and probable left ventricular hypertrophy. The chest roentgenogram (Fig. 3) revealed slight enlargement of the left atrium and left ventricle, but no poststenotic dilatation of the aorta or valvular calcification. Transbronchial left heart catheterization revealed a pressure gradient of 105 mm. Hg between the main left ventricular cavity and the outflow tract. The presence of this systolic pressure gradient below

the aortic valve was confirmed at retrograde left ventricular catheterization. (Fig. 5.) A selective left ventricular angiocardiogram (Fig. 9) revealed a thickened left ventricular wall resulting in a filling defect in the left ventricular cavity. The outflow tract narrowed in characteristic fashion during systole but opened well in diastole. Regurgitation of dye from the left ventricle into the left atrium was also noted.

During the six months since the initial studies the patient has remained asymptomatic and there have been no changes in the clinical findings.

Case VI. G. B. (No. 02-39-78) is a twenty year old white man with a striking family history of heart disease. Both his siblings (Cases VII and VIII) have heart murmurs, and many members of his father's family were said to have heart murmurs. Several of these died suddenly in childhood or during early adult life. No heart murmur was noted on several early examinations but at the age of twelve years, during the course of an infection of the upper respiratory tract associated with a low grade fever, a heart

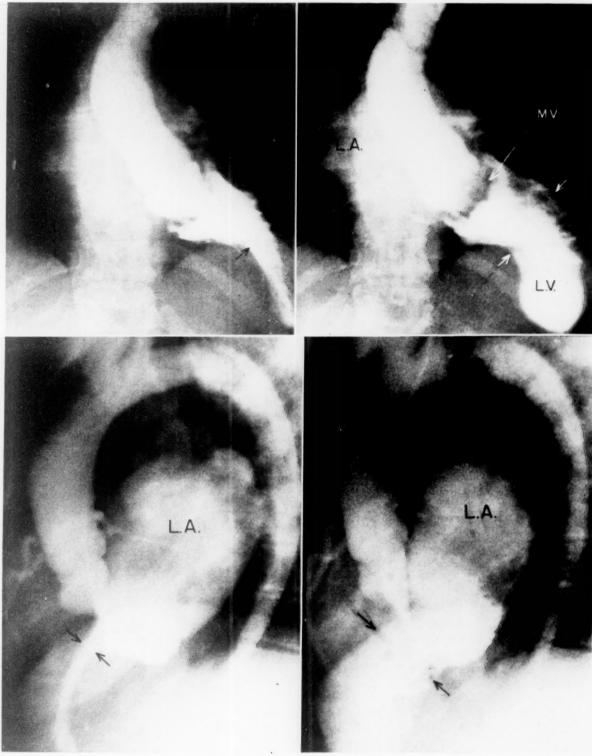


Fig. 6. Case III. Anteroposterior (upper) and lateral (lower) projection angiocardiograms exposed during systole (left) and diastole (right). MV = mitral valve; LA = left atrium; LV = left ventricle. The cone-shaped area of subvalvular obstruction is obscured by the enlarged left atrium, but the marked thickening of the left ventricular wall is evident.

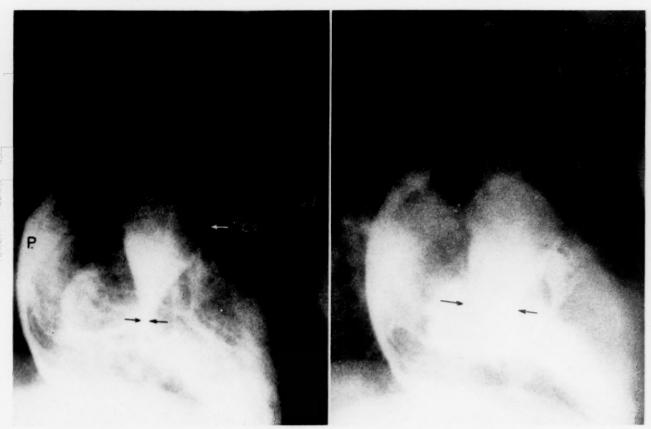


Fig. 7. Case iv. Lateral projection angiocardiograms exposed during systole (left) and diastole (night). P = collection of contrast substance in the pericardium; Ao.V. = aortic valve. Arrows indicate narrowing (left) and widening of the left ventricular outflow tract.

murmur was heard for the first time. The patient had no history of rheumatic fever or of any of its stigmata. At the age of fourteen years he was seen by a prominent cardiologist who detected a grade 3/6 musical late systolic murmur at the cardiac apex and a normal third heart sound. On fluoroscopy slight enlargement of the left ventricle was present. The electrocardiogram showed anomalous atrioventricular excitation and low R waves over the precordium. (Fig. 10.) The diagnosis of rheumatic mitral regurgitation was made, although the possibility of familial heart disease of obscure nature was considered when the same consultant examined his older brother (Case VIII) and noted similar clinical and electrocardiographic findings. Re-examination at the age of eighteen years revealed a grade 2/6 protodiastolic rumble at the apex and a third heart sound, in addition to the pansystolic murmur at the apex. Minimal dyspnea on exertion, which was first noted at the age of thirteen years, progressed very gradually. At admission, the patient was still able to perform light work, climb one and a half flights of stairs, and walk as far as half a mile.

On physical examination the blood pressure was 135/60 mm. Hg and the pulses were full. A prominent left ventricular lift and an apical systolic thrill were

present. A grade 4/6 blowing pansystolic murmur was heard at the apex and along the left sternal

Fig. 8. Electrocardiograms. Top, Case v. Bottom, Case vii.

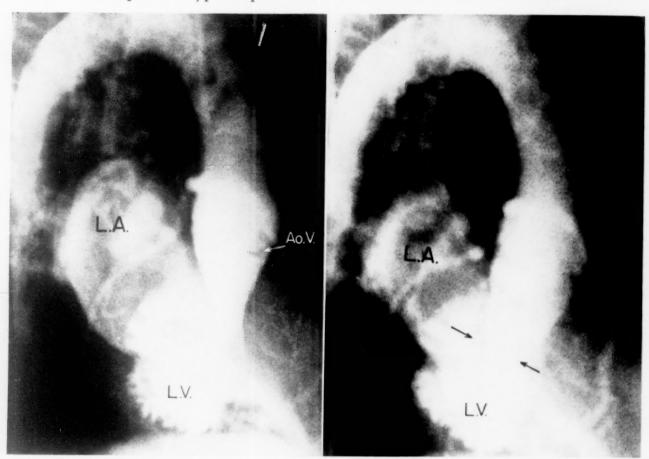


Fig. 9. Case v. Anteroposterior (upper) and lateral (lower) projection angiocardiograms exposed during systole (left) and diastole (right).

border. A short, grade 2/6, early diastolic rumbling murmur was also heard at the apex. The phonocardiogram (Fig. 1) at the apex revealed an atrial sound, followed by a normal first heart sound and a pansystolic murmur. At the left sternal border the delayed aortic component of the second sound was identified by the timing with the incisura of the indirect carotid pulse tracing.

The electrocardiogram (Fig. 10) revealed a normal electric axis and left atrial enlargment; it suggested the presence of left ventricular hypertrophy. On roentgenologic examination (Fig. 3) both the left ventricle and left atrium were enlarged, without evidence of poststenotic dilatation of the aorta or valvular calcification. At right heart catheterization there was no indication of an intracardiac shunt, the pulmonary artery (28/17 mm. Hg) and right ventricular (33/4 mm. Hg) pressures were minimally elevated, and the resting cardiac index was normal (3.47 L./minute/sq. M.). Transseptal left heart catheterization [10] revealed an elevated left atrial pressure (22 mm. Hg), but the contour of the pressure pulse did not suggest mitral regurgitation. A large pressure gradient between the cavity of the left ventricle and the brachial artery was present. This gradient was confirmed by anterior percutaneous left

ventricular puncture, which revealed a 100 mm. Hg gradient and an effective aortic orifice size of 0.25 sq. cm. The brachial artery pressure pulse contour was normal and did not resemble that usually associated with aortic stenosis. (Fig. 4B.) Retrograde arterial left ventricular catheterization localized the obstruction to the outflow tract of the left ventricle. (Fig. 5.) A left ventricular angiocardiogram (Fig. 11) revealed a thickened left ventricular wall with encroachment on the left ventricular cavity. Mild mitral regurgitation was present.

Case VII. S. B. (No. 02-56-06) is a nineteen year old girl and the sister of patients G. B. and C. P. B. (Cases VI and VIII). She was apparently normal at birth and developed normally until the age of six years when she acquired an acute pulmonary infection associated with a temperature of 107°F. Following this illness she was mentally retarded and unable to remain in school. At the age of sixteen years a heart murmur was discovered but she had never evidenced diminished cardiac reserve and had no complaints referable to the cardiovascular system.

On admission, examination revealed marked mental retardation and on psychometric evaluation her I.Q. was 42. The blood pressure was 120/70 mm.

Hg and the peripheral pulses full. The heart was not enlarged but a left ventricular lift and systolic thrill were felt at the apex. A grade 4/6 harsh pansystolic murmur was heard, best at the third interspace 3 cm. to the left of the sternal border. There was paradoxical splitting of the second heart sound. (Fig. 12.) The electrocardiogram (Fig. 8) revealed anomalous atrioventricular excitation. A chest roentgenogram showed slight enlargement of the left atrium and left ventricle. (Fig. 3.) Right heart catheterization demonstrated no evidence of an intracardiac shunt. The pressures in the pulmonary artery wedge position (15 mm. Hg mean) and pulmonary artery (35/13 mm. Hg) were slightly elevated; the cardiac index at rest was at the upper limits of normal (3.65 L./ minute/sq. M.). On retrograde left ventricular catheterization the systolic pressure in the body of the left ventricle was 175 mm. Hg and pulsus alternans was evident. (Fig. 5.) As the catheter was withdrawn, a gradient of 55 mm. Hg was present in the outflow tract of the left ventricle and of 25 mm. Hg across the aortic valve. The left ventricular angiocardiogram revealed elevation and convexity of the medial inferior wall of the left ventricle which encroached on the cavity. The left ventricular outflow tract was narrowed only during portions of the cardiac cycle. No mitral regurgitation was evident

Case viii. C. P. B. (No. 02-51-81), a twenty-two year old white man who is the brother of patients G. B. and S. B. (Cases vi and vii), was admitted to the Clinical Center in September 1959 for diagnostic studies. He was apparently well until the age of eleven years when his physician noted the presence of a heart murmur on a routine examination. He tired easily, had mild dyspnea on severe exertion, and had voluntarily limited his activities for the ten years preceding admission. At the age of sixteen and nineteen years he was seen by a cardiologist who noted a grade 4/6 systolic murmur and a grade 3/6 rumbling diastolic murmur at the apex, as well as a grade 3/6 systolic murmur at the pulmonic area. There was cardiac enlargement on fluoroscopy, with systolic pulsations of an enlarged left atrium, and the electrocardiogram showed anomalous atrioventricular excitation. (Fig. 10.) He had been considered to have rheumatic mitral valvular disease.

Physical examination on admission revealed a blood pressure of 110/60 mm. Hg. The peripheral arterial pulse exhibited a rapid rise and was regular. There was a prominent left ventricular lift and a systolic thrill was palpable between the apex and the lower left sternal border. An atrial sound was audible at the apex. A grade 4/6 blowing ejection systolic murmur was heard at the apex and along the left sternal edge, with some radiation into the axilla. (Fig. 1.) No diastolic murmurs were heard. The indirect carotid pulse tracing showed a rapid ascending limb, and a decline in early systole followed by a

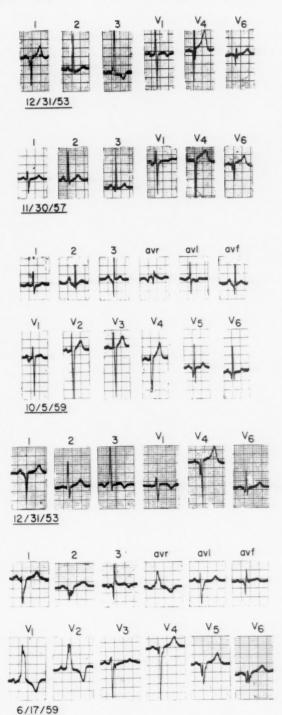


Fig. 10. Electrocardiograms. Top, Case vi. Bottom, Case viii.

second smaller positive deflection; there was no carotid shudder. The electrocardiogram (Fig. 10) showed a broad notched P wave in the standard leads suggesting left atrial enlargement, anomalous atrioventricular excitation with a QRS deformity related to a prolonged and prominent delta wave. Two electrocardiograms taken three months earlier were similar except that the QRS duration was 0.14 second.

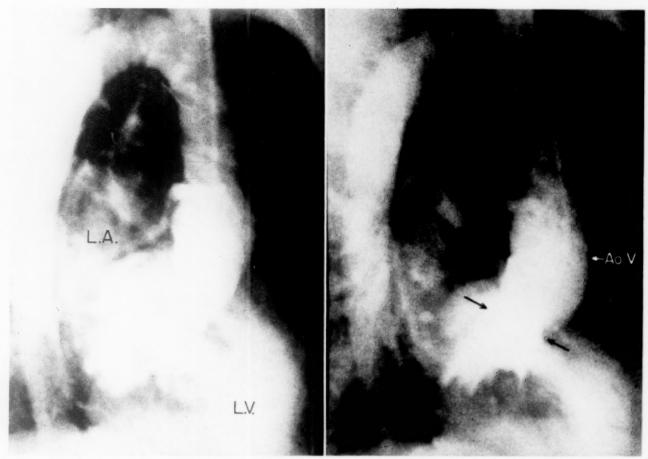


Fig. 11. Case vi. Lateral projection angiocardiogram exposed during systole (left) and diastole (right),

A chest roentgenogram (Fig. 3) revealed cardiac enlargement with prominence of the left ventricle, no poststenotic dilatation of the aorta, and no valvular calcification.

Right heart catheterization revealed mild pulmonary hypertension (pulmonary artery, 35/10 mm. Hg at rest and 54/26 during exercise), no evidence of an intracardiac shunt, a low cardiac index at rest (2.17 L./minute/sq. M.) with an inadequate rise with exercise. A retrograde left ventricular catheterization revealed pulsus alternans within the left ventricle, a gradient of 40 mm. Hg across the outflow tract of the left ventricle and of 10 mm. Hg across the aortic valve. Atrial contraction resulted in an elevated end diastolic pressure of 32 mm. Hg, and a notch on the upstroke of the left ventricular pressure was present proximal to the obstruction. (Fig. 5.) As preparations were being made to perform a selective left ventricular angiocardiogram, and while the catheter tip was in the arch of the aorta, ventricular fibrillation suddenly occurred. Thoracotomy and cardiac massage were performed immediately and the heart defibrillated easily. The patient's course after thoracotomy was uneventful except for a temporary increase in prominence of the protodiastolic gallop.

Case IX. C. F. (No. 02-80-79), a ten year old boy, had a heart murmur discovered at the age of three months. There was no family history of heart disease. At four years of age he was examined by a cardiologist who made the clinical diagnosis of ventricular septal defect. Because of increasing fatigability he was referred to the University of Saskatchewan, Canada, at the age of eight years, where a clinical diagnosis of subvalvular aortic stenosis was made. Right heart catheterization at that time revealed no evidence of a left-to-right or a right-to-left shunt, and the pulmonary artery pressure was slightly elevated (30/15 mm. Hg).

Because of the presence of cardiac enlargement, fatigability and dyspnea, the patient was admitted to the National Heart Institute on January 10, 1960. The pertinent findings on physical examination were limited to the cardiovascular system. The peripheral pulses were brisk and regular, and the blood pressure 85/55 mm. Hg. A prominent left ventricular lift was palpable, and the heart was enlarged, with the point of maximum impulse in the sixth intercostal space in the anterior axillary line. A systolic thrill was felt over the entire precordium. The second heart sound was paradoxically split at the base (Fig. 12) and there was a grade 4/6 ejection systolic murmur heard well

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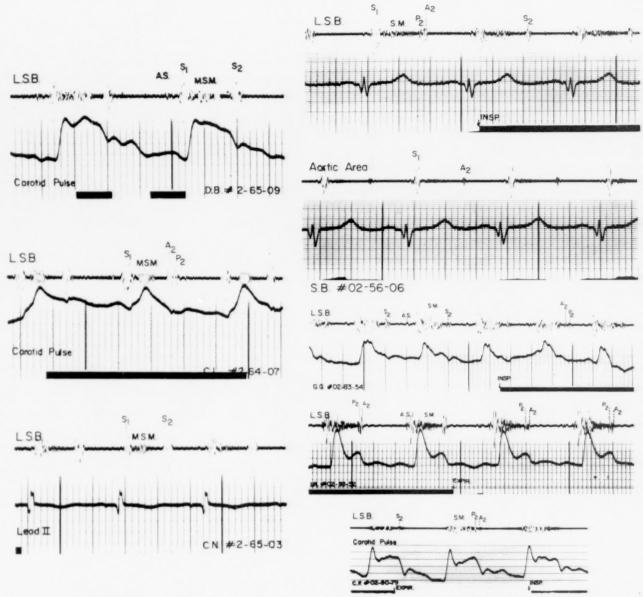


Fig. 12. Phonocardiograms. MSM = mid systolic murmur. Other symbols same as Figure 1. Reading from top to bottom. Left, the records obtained on three relatives of patients G. B., S. B. and C. P. B. (Cases VI, VII and VIII), as discussed in the text. Right, the upper two tracings are from patient S. B. (Case VII); the third tracing is from patient G. S. G. (Case X); the fourth tracing is from patient I. R. (Case XI); the bottom tracing is from patient C. F. (Case IX).

over the entire precordium, but not transmitted to the neck. The electrocardiogram showed first degree A-V block, left ventricular hypertrophy, with T wave inversion and S-T segment depressions in the standard leads and over the left precordium. The chest roentgenogram (Fig. 3) showed marked cardiac enlargement, predominantly of the left ventricle. There was no dilatation of the ascending aorta.

On the basis of the clinical diagnosis of idiopathic hypertrophic subaortic stenosis a retrograde left ventricular catheterization was carried out. The aortic pressure showed a brisk rise, and there was no pressure gradient across the aortic valve. However, the pressure within the left ventricular cavity was 260/20 mm. Hg and there was a 185 mm. Hg systolic pressure gradient in the left ventricular outflow tract. (Fig. 13.) The left ventricular angiocardiogram again showed marked narrowing of the left ventricular outflow tract during portions of the cardiac cycle, with relaxation of this area at other times.

On January 26, 1960, the patient was operated upon with the aid of total cardiopulmonary bypass. After caval cannulation, but before bypass was established, a catheter was introduced from the apex of the left ventricle and confirmed the presence of a left intraventricular pressure gradient (80 mm. Hg). An incision in the aorta revealed the aortic valve to be normal. Below the valve ring there was visible a

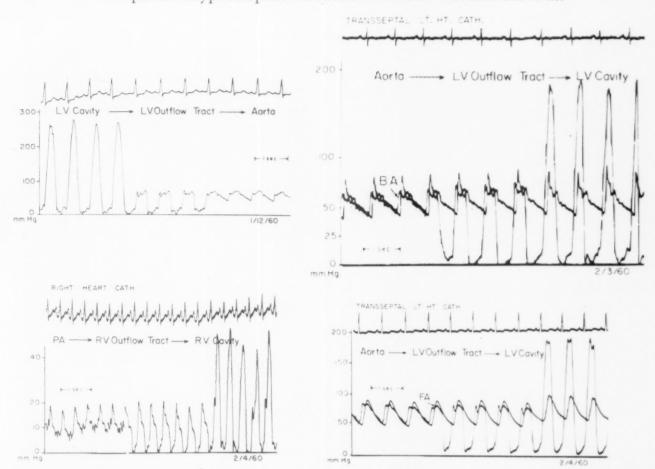


Fig. 13. Continuous pressure recording in the left ventricular cavity, the left ventricular outflow tract and aorta. *Upper left*, Case IX. *Upper right*, Case IX. *Lower right*, Case IX. *Lower left*, same case, pressure recording from the pulmonary artery, right ventricular outflow tract, and right ventricular cavity.

dome-shaped area of left ventricular hypertrophy, which seemed to involve primarily the septal portion of the left ventricular outflow tract. Attempts to remove portions of the left ventricular muscle with a Brock punch were unsatisfactory. A longitudinal incision was made through the endocardium and in the ventricular muscle from near the apex of the ventricle to the aortic annulus. The incision was in the anterior wall. The muscle fibers were then split with the finger to a depth of 2.5 cm. Following the termination of cardiopulmonary bypass, repeat measurements showed that the left intraventricular pressure gradient had been reduced to 35 mm. Hg, at a time when the blood pressure remained well maintained.

The patient's postoperative convalescence was prolonged but he made a satisfactory recovery. The left ventricular systolic pressure, measured by percutaneous puncture, was 160 mm. Hg five weeks after the operation and the peak systolic gradient was found to have been reduced to 60 mm. Hg.

Case x. G. S. G. (No. 02-83-54) is a six year old boy who was admitted to the National Heart Institute

on January 31, 1960. At the age of two weeks a heart murmur was discovered and he had been followed since that time with a tentative diagnosis of ventricular septal defect. He remained asymptomatic, except for easy fatigability, but developed progressive left ventricular enlargement evident both on the electrocardiogram and the roentgenogram.

Physical examination on admission showed that his pulses were brisk. The blood pressure was 110/80 mm. Hg. Both right and left ventricular lifts were palpable and a systolic thrill was felt along the left sternal border, maximal at the third intercostal space. The second heart sound exhibited normal splitting during the respiratory cycle. A grade 4/6 ejection systolic murmur was present over the entire precordium (Fig. 12); it was loudest at the second and third left intercostal spaces, but did not radiate to the neck. The electrocardiogram showed left ventricular hypertrophy with rotation of the T wave vector rightward and posteriorly. The roentgenogram (Fig. 3) revealed cardiac enlargement, primarily of the left ventricle, but the left atrium and right ventricle were also thought to show some enlargement. The clinical diagnosis of ventricular septal defect was questioned

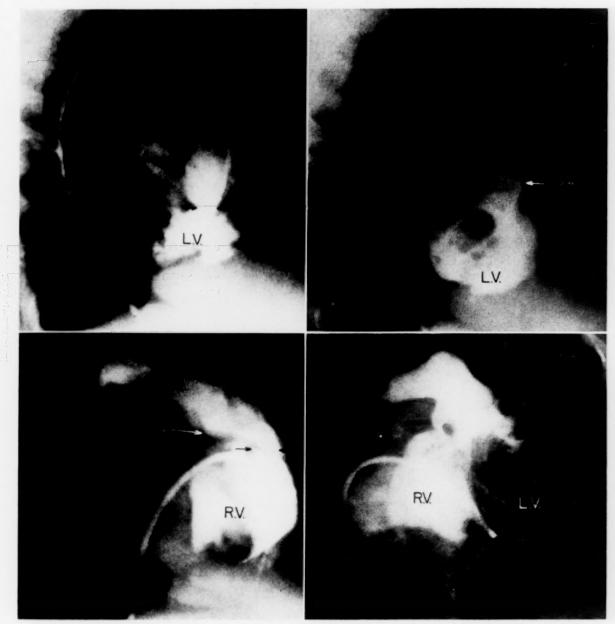


Fig. 14. Lateral exposure of left ventricular angiocardiogram during systole (upper left) and diastole (upper right). Right ventricular angiocardiogram, lateral exposure (left) and anteroposterior exposure (right).

because the murmur was not pansystolic and because of the markedly abnormal T wave vector. Right heart catheterization revealed no evidence of any circulatory shunts. The pressure in the main right ventricular cavity was elevated (50/5 mm. Hg), but it was normal (21/14 mm. Hg) in the pulmonary artery. Withdrawal of the catheter showed that the systolic pressure gradient was in the right ventricular outflow tract. (Fig. 13.) A selective angiocardiogram with right ventricular injection showed the right ventricle to be displaced anteriorly and to the right, presumably by an enlarged left ventricle. Although there was no discrete area of stenosis within the right

ventricular outflow tract, this entire region appeared to be narrowed. (Fig. 14.)

Because of the suspicion of the diagnosis of idiopathic hypertrophic subaortic stenosis, transseptal left heart catheterization was carried out. A 95 mm. Hg pressure gradient was demonstrated in the left ventricular outflow tract. (Fig. 13.) A similar gradient was discovered at the time of retrograde catheterization of the left ventricle; the left ventricular angiocardiogram (Fig. 14) showed that the thickened left ventricular muscle encroached upon the left ventricular cavity. A narrowed area several centimeters below the aortic valve varied in size during the cardiac cycle. A mild degree of mitral regurgitation was also present.

CASE XI. I. R. (No. 02-83-52) is a thirty-three year old man whose family history of heart disease was negligible and who was apparently well until the age of twenty-seven years. At that time he had epigastric pain on exertion, which radiated to the chest and neck. In addition he had frequent episodes of dizziness and fainted several times. Physical examination at the University of Saskatchewan at that time revealed an ejection systolic murmur loudest between the apex and the lower left sternal border, and evidence of left ventricular hypertrophy on the electrocardiogram and roentgenogram. Right heart catheterization at this time revealed no abnormalities. Transbronchial left heart catheterization, carried out by Dr. E. M. Nanson, revealed a 61 mm. Hg systolic pressure gradient between the left ventricle and the brachial artery. At operation, which was performed under hypothermia by Dr. Nanson, a cannula was inserted into the left ventricle for the measurement of pressures; there was no systolic pressure gradient across the aortic valve, but 3 cm. below the valve a 40 mm. Hg systolic pressure gradient was recorded. It was considered that this represented an unusual type of subaortic stenosis and relief of the obstruction was not attempted.

Because of the continuation of his symptoms the patient was admitted to the Mayo Clinic in April 1957. Pressure records from the radial artery and aorta did not suggest the presence of aortic stenosis. At operation by Dr. F. Henry Ellis, Jr. there was a thrill over the left atrium, but none over the aorta. Palpation within the left atrium revealed grade 2 mitral regurgitation. Systolic pressures measured within the left ventricular cavity and the aorta were identical and the chest was closed. Following this operation the patient did reasonably well until November 1959 when severe dyspnea, orthopnea and paroxysmal nocturnal dyspnea developed and he required intensive treatment for congestive heart failure. He was referred to the National Heart Institute by Drs. Nanson and Horlick with the diagnosis of idiopathic hypertrophic subaortic stenosis.

Examination at the time of admission to the National Heart Institute (January 1960) revealed a blood pressure of 110/60 mm. Hg, and brisk peripheral pulses. There was a prominent left ventricular lift in the sixth intercostal space at the anterior axillary line. A systolic thrill was present at the apex only. There was paradoxical splitting of the second heart sound, an atrial sound was audible, and a grade 4/6 harsh, long, systolic murmur was present at the apex. (Fig. 12.) X-ray examinations revealed enlargement of the left ventricle, but there was no evidence of calcification in the region of the aortic valve or of poststenotic dilatation. The electrocardiogram showed left ventricular hypertrophy with T wave inversions

and S-T segment depressions in the standard leads and over the left precordium. At right heart catheterization the pulmonary artery pressure was 27/8 mm. Hg. There was no systolic pressure gradient in the right ventricular outflow tract and no evidence of a cardiac shunt. Transseptal left heart catheterization showed a left ventricular pressure of 190/15 mm. Hg and a 70 mm. Hg gradient within the left ventricular outflow tract. (Fig. 13.) The cardiac output was 3.94 L./minute and the effective orifice size within the ventricle 0.50 sq. cm.

Operation was carried out with the aid of total cardiopulmonary bypass on February 16, 1960. The aorta was of normal size, the aortic valve was entirely normal and there was no evidence of discrete subaortic stenosis. After the aorta had been opened a long diffuse area of grossly thickened and hypertrophic muscle was evident in the left ventricular outflow tract, more prominently on the septal surface. A vertical incision was made in the endocardium from the ventricular apex to the aortic annulus and the muscle fibers were then split with the finger until the incision had been carried down at least 2 cm. into the substance of the muscle. The patient's postoperative course has been normal. The left ventricular systolic pressure, measured by percutaneous puncture three weeks after operation, was 145 mm. Hg and the peak systolic gradient at this time was 30 mm. Hg. The effective orifice size was 1.61 sq. cm.*

COMMENTS

It is now well recognized that the muscular hypertrophy which sometimes develops in patients with severe stenosis of either the pulmonic or the aorta valve may narrow the ventricular outflow tract and, of itself, offer significant obstruction to ventricular ejection. Brock has pointed out that such deformity of the right ventricle may not be recognized before operation because the elevated intraventricular systolic pressure distends the subvalvular region and prevents the hypertrophied muscular area from approximating during systole [11]. However, this process assumes greater physiologic importance immediately after pulmonic valvotomy when the lowered right ventricular systolic pressure permits the thickened outflow tract to constrict during systole; a large subvalvular pressure gradient then develops [11–15]. Thus, despite complete anatomic relief of valvular obstruction the patient may at first

^{*} Since submission of this manuscript we have studied three other patients with idiopathic hypertrophic subaortic stenosis. Brief descriptions of these patients are presented in the Addendum.

realize little benefit since the right ventricular systolic pressure is not substantially reduced. Fortunately, this muscular hypertrophy of the outflow tract gradually recedes after the stimulus for its formation, the narrowed pulmonic orifice, has been removed.

Rodbard and Shaffer [1] analyzed the pressure pulses recorded from the right ventricular inflow and outflow tracts in patients with infundibular pulmonic stenosis and suggested that two forms of this malformation exist. In three patients the right ventricular and infundibular pressure tracings had similar phase relationships, with simultaneous peaks. It was postulated that in these patients the stenotic orifice between the two chambers has a fixed cross sectional area and is characterized anatomically by a fibrous band in the infundibulum. In ten patients, however, the systolic peak of the pressure pulse immediately beneath the valve preceded the peak pressure in the main right ventricular cavity. In these patients, the authors suggested progressive contraction of the subvalvular muscular ring during systole narrowed the stenotic orifice and thereby lowered the pressure distal to the obstruction in late systole. The presence of such a muscular ring was subsequently confirmed at operation in two patients [1,16]. The hemodynamic observations of Harris [17] and of Bassett [18] also support these views of Rodbard.

In a similar manner Johnson [8] analyzed the right ventricular, infundibular and pulmonary artery pressure pulses in patients with valvular pulmonary stenosis and secondary hypertrophic infundibular stenosis. This investigator suggested that (1) the contraction of the infundibulum commences approximately 0.1 second after the body of the ventricle, (2) infundibular contraction is maximal at the time of right ventricular protodiastole, (3) complete anatomic separation of the right ventricle and its outflow tract occurs at this time, and (4) the prolongation of right ventricular systole in atrial septal defect may be explained by a similar mechanism.

It has been postulated that similar muscular hypertrophy may occur in the outflow tract of the left ventricle in patients with valvular or discrete subvalvular aortic stenosis [2]. Indeed, in one of our patients with congenital subvalvular stenosis, serial left heart catheterizations demonstrated the hemodynamic importance and regression of this hypertrophic type of subaortic stenosis in the nineteen months follow-

ing open operation [4]. Brock [5] has also demonstrated the development of a subvalvular gradient following the relief of valvular obstruction. In a careful hemodynamic and pathologic study of a patient in whom the clinical picture of aortic stenosis developed coincident with spontaneous reduction of long standing systemic arterial hypertension, Brock indicated that such "functional aortic stenosis" may result from ventricular hypertrophy with an etiology other than valvular aortic stenosis [2].

More recently, Brock described five other adult patients without systemic hypertension in whom ventricular hypertrophy resulted in "functional" obstruction to left ventricular outflow [5]. In 1958 Bercu and his collaborators [3] reported a patient who was operated upon for valvular aortic stenosis after a large systolic pressure gradient was detected between simultaneously recorded left ventricular and systemic arterial pressure pulses. At open operation no discrete obstruction was encountered; at postmortem examination massive symmetrical hypertrophy of both ventricular walls, with striking reduction in the size of both ventricular cavities, was evident. In their recent comprehensive review of subaortic stenosis Brachfeld and Gorlin [6] called attention to the muscular hypertrophy of the subvalvular region which frequently accompanies both congenital aortic valvular stenosis and the discrete subvalvular membrane of congenital subaortic stenosis. These authors emphasized the functional importance of this muscular sphincter and postulated that its contraction during ventricular systole might offer an impediment to left ventricular emptying. Two of their patients had anatomic evidence of a discrete subvalvular diaphragm of fibrous tissue as well as bulging of hypertrophied myocardium into the ventricular outflow tract. In their other two patients, however, myocardial hypertrophy apparently was the primary cause of obstruction. It seems likely, on the basis of clinical, hemodynamic and anatomic similarities, that the fourteen patients described in the present report, the latter two patients described by Brachfeld and Gorlin [6], the patients of Bercu [3], of Brent and associates [7], and all but the first of Brock's patients [2,5] constitute a distinct entity.

Pertinent to any consideration of the etiology of the muscular hypertrophy responsible for the obstruction to left ventricular outflow in idiopathic hypertrophic subaortic stenosis is the

study by Teare [19] in which the autopsy descriptions of the hearts of eight patients, ranging in age from fourteen to forty-five years, are presented. Unfortunately, relatively sparse clinical information and no hemodynamic data were available in these patients. Seven of these patients died suddenly and the eighth died after an attempted mitral valvotomy. At postmortem examination there was diffuse hypertrophy of the interventricular septum in all patients, with some extension of this process to the anterior wall of the left ventricle in several instances. Microscopic examination revealed a bizarre arrangement of bundles of muscle fibers, the latter exhibiting considerable variation in size and some being separated by clefts lined with endothelium. It was observed that the asymmetric ventricular hypertrophy resulted in distortion of an otherwise normal mitral valve in one heart and extended into close proximity of the valve in another. Teare considered the abnormality to be developmental in origin. It is possible that left ventricular hypertrophy of unknown origin, similar to if not identical with that described by Teare, is responsible for the clinical, hemodynamic and angiocardiographic findings in idiopathic hypertrophic subaortic stenosis. Indeed, the thin, slit-like, left ventricular cavities in the specimens illustrated by Teare [19] are certainly reminiscent of the left ventricular angiocardiograms of our patients. It is not difficult to imagine that further reduction in the size of these ventricular cavities, occurring during ventricular systole, would result in obstruction to ventricular emptying.

A mild to moderate degree of mitral regurgitation was demonstrated either at operation or by the reflux of contrast substance from the left ventricle into the left atrium in 11 of our 12 patients studied in this manner. However, in the patients operated upon, the mitral valve leaflets were normal to palpation. The distortion of the mitral valve by the hypertrophied ventricular septum, as demonstrated by Teare [19], is probably responsible for the reflux. The mitral stenosis present in patient M. H. (Case III) (Fig. 4A) may be caused by this process also.

It is evident that in some instances idiopathic hypertrophic subaortic stenosis may be familial. Thus two siblings described by Teare [19] had asymmetric ventricular hypertrophy on pathologic examination. Clinical examination of this family revealed that nine of twenty-three persons had unequivocal evidence of heart disease and

another three had possible evidence [20]. A closed transventricular aortic valvulotomy, for what was believed to be valvular aortic stenosis, had been performed on the brother of the patient described by Bercu and associates [3]. Idiopathic hypertrophic subaortic stenosis was proved at postmortem examination in three members of two families studied by Brent and associates [7]. Clinical examination in several other members in these two families suggests that a similar process may be present.

Three of the fourteen patients described in this report are siblings. Examination of both parents of these three patients revealed no evidence of heart disease but in view of a history of heart murmurs with several unexplained sudden deaths among the paternal relatives, further attention was directed to this branch of the family. Seventy-five persons, most of whom were first or second cousins of the propositi, were examined. Three, two boys aged four and six years, and a forty-seven year old woman, had distinctly abnormal physical findings even though they were asymptomatic. All had prominent left ventricular lifts and grade 3 harsh systolic ejection murmurs along the left sternal border. (Fig. 12.) Although the electrocardiograms in all three were normal, the roentgenograms suggested left ventricular enlargement. Simultaneous left ventricular and brachial artery pressures revealed no systolic pressure gradient in the two boys, and the results of right heart catheterization in the woman were within normal limits. Patients M. O'D. and T. O'D. (Cases XIII and XIV) are mother and son. Furthermore, although we have not studied them, the history suggests that one sister of patient M. O'D. (Case XIII) also has idiopathic hypertrophic subaortic stenosis and that another sister died of this condition.

The observation of anomalous atrioventricular excitation in the three siblings with idiopathic hypertrophic subaortic stenosis supports the concept [21] that, in some instances at least, this electrocardiographic finding may be familial.

With the delineation of the clinical, hemodynamic, angiographic and anatomic manifestations of the patients with this malformation reported herein and elsewhere [2–7], idiopathic hypertrophic subaortic stenosis emerges as a specific disease process. It is certainly possible, however, that with continued experience several varieties of idiopathic hypertrophic subaortic stenosis will be recognized. In some patients,

as already indicated, a strong familial association with suggestive evidence of a Mendelian dominant inheritance [7] is present. In the majority of patients, however, no familial association is evident. In most instances, idiopathic hypertrophic subaortic stenosis appears to be a lesion which develops in adolescence or adult life and is progressive in nature. Several of the patients described herein were examined in childhood and no evidence of heart disease was detected. The development, in adult life, of a heart murmur and then of left ventricular hypertrophy in patient D. E. (Case IV) is well documented and is particularly noteworthy. On the other hand, in the three youngest patients in this series (Cases ix, x and xii) heart murmurs were detected in infancy. It is possible that the left ventricular hypertrophy was congenital in these two patients. The diffuse nature of their obstruction to left ventricular outflow makes their lesion anatomically and physiologically distinct from the usual, discrete form of subvalvular aortic stenosis. Thus it is possible that three subtypes of this disease process may exist: (1) The familial, non-congenital variety seen in patients of all ages (Cases vi, vii, viii, xiii and xiv of this report); (2) the non-congenital variety seen in adults without evidence for any familial association (Cases I, II, IV, V and XI); and (3) the non-familial congenital variety (Cases IX, X and XII).

Previous descriptions of patients have named this disease process "functional aortic stenosis" [2,4,5], "pseudoaortic stenosis" [3], and "familial muscular subaortic stenosis" [7]. It is now clear that a true organic basis for the obstruction is provided by the hypertrophied muscular outflow tract, making the term "functional" a misnomer. Also, since a specific cause for the left ventricular hypertrophy has not been apparent in any patient and a familial association could be demonstrated in only a fraction of them, the phrase "idiopathic hypertrophic subaortic stenosis" is suggested as the most appropriate designation for this lesion.

The severity of idiopathic hypertrophic subaortic stenosis varies widely, the malformation in the mildest cases resulting only in a prominent systolic ejection murmur along the left sternal border accompanied by evidence of mild left ventricular hypertrophy on clinical and roentgenologic examination. In these patients the narrowing of the outflow tract is not severe enough to produce a systolic pressure gradient.

In other patients with a somewhat more advanced form of this process, a definite systolic pressure gradient is present but the obstruction may not be severe enough to cause symptoms. When definite cardiovascular symptoms are present a large systolic pressure gradient has always been found. The symptoms are not specific; palpitations, fatigability and exertional dyspnea occur frequently; angina, syncope and congestive heart failure have also been noted. Sudden death may occur in both symptomatic

and asymptomatic patients [7,19].

On physical examination the patients with idiopathic hypertrophic subaortic stenosis are well developed and no congenital anomalies have been noted, even in those with the familial form of this disease. The physical findings classically associated with discrete valvular, subvalvular or supravalvular aortic stenosis are not evident. In most instances the diagnoses of ventricular septal defect or of mitral insufficiency have been suspected clinically. In patients with moderate or severe obstruction to left ventricular ejection, examination of the chest reveals a prominent apical thrust just lateral to the midclavicular line; a left ventricular lift is easily felt, and a systolic thrill is palpable over the lower precordium, extending from the apex to the left sternal border. The thrill is rarely felt in the jugular notch or along the carotid vessels. The peripheral pulses are normal or even brisk to palpation and the prolonged upstroke characteristic of severe valvular aortic stenosis is not present. The rhythm is generally regular although atrial fibrillation may occur. An atrial sound is heard or recorded frequently and indicates that the resistance to left ventricular filling is increased. A loud, harsh, long systolic murmur is heard over the entire precordium but is most prominent at the apex and along the lower left sternal border. This murmur may be transmitted into the axilla, and in the majority of patients is not heard well in the second right intercostal space or along the carotid vessels. The first heart sound is within normal limits but the sound of aortic valve closure is delayed in patients in whom a large intraventricular pressure gradient is present. Paradoxical splitting of the second heart sound [22] may be readily appreciated either by auscultation or on the phonocardiogram. In some patients with delayed aortic valve closure the sound of pulmonary valve closure is obscured by the long systolic murmur. A simultaneous recording of the phonocardio-

gram and of the carotid pulse tracing reveals that the single second heart sound is the sound of delayed aortic valve closure. Atrial or ventricular gallop rhythms are frequently audible. but the high-pitched decrescendo diastolic murmur along the left sternal border, which is associated with aortic valve regurgitation, has not been heard in patients with idiopathic hypertrophic subaortic stenosis; its presence should make the physician question this diagnosis. The indirect carotid pulse tracing does not reveal the anacrotic shoulder and vibrations characteristic of aortic stenosis. Instead, the upstroke is sharp, with a cut-off in mid-systole followed by a second, smaller positive deflection.

Roentgenographic examination reveals mild to moderate enlargement of the cardiac silhouette. The left ventricle is rounded and has the contour which is characteristically associated with concentric hypertrophy, as in valvular aortic stenosis. The left atrium is frequently enlarged, particularly when mitral regurgitation is also present, but the right side of the heart is not particularly prominent. The absence of valvular calcification and of dilatation of the ascending aorta are important roentgenographic findings. The electrocardiogram also shows left ventricular hypertrophy, and in some patients left atrial enlargement. Anomalous ventricular excitation has been noted in four of our patients. Right heart catheterization occasionally reveals a small systolic pressure gradient in the outflow tract of the right ventricle (Fig. 13); the pulmonary artery pressure is either normal or slightly elevated. The resting cardiac output is normal or slightly depressed at rest, and generally fails to rise in a normal fashion with exercise. The mean left atrial pressure is usually slightly elevated and the contour of the pressure pulse does not suggest the presence of gross mitral regurgitation even in patients in whom some evidence for this is subsequently found at operation or by means of left ventricular angiography. The atrial contraction waves ("a" waves) are prominent and the left atrial pressure at the end of ventricular diastole, the "z point pressure," exceeds the mean left atrial pressure.

A systolic pressure gradient between the main cavity of the left ventricle and a systemic artery is necessary for the diagnosis of hemodynamically significant idiopathic hypertrophic subaortic stenosis. In those instances in which the catheter cannot be manipulated across the aortic valve, or when left ventricular pressure is measured simply by means of anterior percutaneous puncture, the combination of a large systolic pressure gradient associated with a normally rapid ascending limb of the brachial artery pressure pulse [6] should suggest the diagnosis. The arterial pressure tracings were not suggestive of severe aortic stenosis in any of our patients; the duration of the systolic upstroke ranged from 0.09 to 0.12 second. In this connection, it is of interest that in the patients with valvular aortic stenosis studied by Wood [23], the brachial artery upstroke durations ranged from 0.13 to 0.29 and averaged 0.23 second.

Hemodynamic localization of the site of obstruction is afforded only by recording pressure pulses sequentially from the aorta, the left ventricular outflow tract and the main body of the left ventricle. Pulsus alternans is frequently present in the recordings from the left ventricular cavity proximal to the obstruction. This hemodynamic phenomenon occurs very infrequently in patients with the congenital forms of aortic stenosis [24]. The pressure pulse in the outflow tract of the left ventricle distal to the obstructing muscular lesion usually peaks in early ventricular systole, reflecting the progressively increasing obstruction during ventricular contraction [1,6]. The prominent left atrial contraction wave, mentioned previously, is transmitted into the left ventricle and results in a markedly elevated left ventricular end diastolic pressure. This phenomenon may well be related to the decreased compliance of the hypertrophied left ventricle. The ascending limb of the pressure pulse recorded from the left ventricular cavity proximal to the obstruction generally exhibits a notch or pause at a level which corresponds to the peak pressure distal to the obstruction. A similar finding was present in a tracing published by Brachfeld and Gorlin [6] and it may signify the delayed onset of contraction of the sphincteric outflow tract. Although these left ventricular pressure measurements may be carried out by means of a catheter introduced by way of the left atrium, retrograde left ventricular catheterization through a systemic artery [25] has been found particularly useful in studying patients with suspected idiopathic hypertrophic subaortic stenosis, since a left ventricular angiocardiogram may be conveniently performed immediately following demonstration of the subvalvular pressure gradient.

Selective left ventricular angiocardiography constitutes an important diagnostic technic in the study of patients with this disease. A relatively narrow left ventricular cavity is usually demonstrated, the aortic valve appears normal, there is no dilatation of the ascending aorta and some reflux of dve into the left atrium is frequently apparent. Of greater importance, however, is the demonstration of the marked thickening of the left ventricular wall which actually encroaches on the inferior portion of the cavity. During phases of the cardiac cycle the outflow tract narrows even further, but it expands during the remainder of the cycle. In the lateral view the angiocardiogram shows the ventricular outflow tract in the shape of an inverted cone with the base at the aortic valve. When the muscular outflow tract contracts, the cone comes to a sharp point several centimeters below the aortic valve; when the outflow tract relaxes, the cone appears to be truncated.

The recognition of idiopathic hypertrophic subaortic stenosis, and its distinction from those forms of aortic stenosis with a discrete site of obstruction which are readily amenable to surgical correction, would appear to be essential for formulating a plan for treatment. Although the clinical and hemodynamic results of the operations performed in Cases 1x and x1 (which was suggested to one of us [A. G. M.] by Mr. W. P. Cleland) are certainly encouraging, an evaluation of the ultimate effectiveness of this procedure and relative risk will require considerably more experience and a longer period of follow-up. At the present time, however, it would appear appropriate to attempt to relieve the muscular obstruction only in those patients who are progressively symptomatic and in whom a large intraventricular pressure gradient can be demonstrated.

SUMMARY

The diagnosis of idiopathic hypertrophic subaortic stenosis, i.e., left ventricular hypertrophy producing severe obstruction to left ventricular outflow, has been established in fourteen patients. All had systolic murmurs, most prominent either at the mitral or tricuspid areas. Left ventricular systole was prolonged, resulting in paradoxical splitting of the second heart sound. Electrocardiograms showed either left ventricular hypertrophy or anomalous atrioventricular excitation, and roentgenograms demonstrated left atrial and ventricular enlargement without aortic dilatation. Left heart catheterization revealed systolic pressure gradients within the ventricle ranging from 40 to 185 mm. Hg, and localized the site of obstruction to the left ventricular outflow tract in every patient. The left ventricular pressure pulses exhibited a characteristic notch in early ventricular systole, and a striking rise during atrial contraction. Both the palpable and recorded peripheral arterial pressure pulses rose rapidly during early systole, unlike those in valvular aortic stenosis [6]. Left ventricular angiocardiograms demonstrated a markedly thickened ventricular wall obstructing the outflow tract of the left ventricle only during a portion of the cardiac cycle.

At open operation in five patients the angiocardiographic interpretations were confirmed; in two patients the hypertrophied muscular ring was incised longitudinally to relieve the obstruction and the early clinical and hemodynamic results of this operation are encouraging.

With the delineation of its clinical, hemodynamic, angiocardiographic and anatomic features, idiopathic hypertrophic subaortic stenosis emerges as a specific entity which can be distinguished preoperatively from discrete valvular and subvalvular aortic stenosis. Its recognition is important in selecting for operation patients with all forms of obstruction to left ventricular outflow and in planning the surgical procedure.

ADDENDUM

CASE XII. J. P. (No. 03-00-93), a seven year old boy, had a heart murmur noted at four months of age and had remained asymptomatic. The family history was non-contributory. On examination the pulses were brisk, and an enlarged left ventricle was palpable. The second heart sound at the base exhibited paradoxical splitting. A prominent systolic thrill and grade 4/6 holosystolic murmur were most prominent just inside the apex. The electrocardiogram showed left ventricular hypertrophy and the roentgenogram revealed left atrial and left ventricular hypertrophy. Right heart catheterization showed no abnormalities. Transseptal left heart catheterization revealed a mean left atrial pressure of 12 mm. Hg with an "a" wave of 18 mm. Hg. The left ventricular pressure was 175/20 mm. Hg with a systolic pressure gradient of 55 mm. Hg in the left ventricular outflow tract. The left ventricular angiocardiogram showed a mild degree of mitral regurgitation, and thickening of the medial, inferior and posterior surfaces of the left ventricular wall. Systolic narrowing of the left ventricular outflow tract was seen 3.5 cm. below the aortic valve.

CASE XIII. M. O'D. (No. 03-15-68), a forty-five year old white woman, the mother of patient T. O'D. (Case xiv), had a heart murmur detected at the age of ten years. Following this she noted fatigability and dyspnea on severe exertion. These symptoms progressed and for four years she experienced several episodes of left and right heart failure as well as angina pectoris. On examination there was a left ventricular lift, a fourth heart sound at the apex, and a grade 3/6, harsh, ejection systolic murmur along the left sternal border; the second heart sound was single. The electrocardiogram and roentgenogram revealed left ventricular hypertrophy; no valvular calcification or aortic dilatation were evident. Right heart catheterization showed slight elevation of the pulmonary artery pressure (35/15 mm, Hg) and retrograde left ventricular catheterization revealed a 95 mm. Hg gradient in the left ventricular outflow tract. The left ventricular angiocardiogram showed mild mitral regurgitation, left ventricular thickening and the systolic narrowing of the left ventricular outflow tract characteristic of idiopathic hypertrophic subaortic stenosis.

Case xiv. T. O'D. (No. 03-16-61), a thirteen year old boy, the son of patient M. O'D. (Case XIII), had a heart murmur detected at the age of five years, although examinations in earlier childhood had not revealed any abnormalities. He had remained entirely asymptomatic. On examination he was obese and had no evidence of cardiac enlargement. A grade 3/6 ejection systolic murmur was heard best just within the cardiac apex. The electrocardiogram showed left ventricular hypertrophy but the roentgenogram was normal. The results of right heart catheterization were within normal limits. Left heart catheterization revealed a 90 mm. Hg gradient in the left ventricular outflow and the left ventricular angiocardiogram showed hypertrophied left ventricular muscle encroaching on the left ventricular outflow tract during ventricular systole, with widening of the outflow tract during diastole.

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REFERENCES

1. Rodbard, S. and Schaffer, A. B. Muscular contraction in the infundibular region as a

- mechanism of pulmonic stenosis in man. Am. Heart J., 51: 885, 1956.
- BROCK, R. Functional obstruction of the left ventricle. (Acquired aortic subvalvular stenosis.) Guy's Hosp. Rep., 106: 221, 1957.
- Bercu, B. A., Diettert, G. A., Danforth, W. H., Pund, E. E., Jr., Ahlvin, R. C. and Belliveau, R. R. Pseudoaortic stenosis produced by ventricular hypertrophy. Am. J. Med., 25: 814, 1958.
- Morrow, A. G. and Braunwald, E. Functional aortic stenosis. A malformation characterized by resistance to left ventricular outflow without anatomic obstruction. *Circulation*, 20: 181, 1959.
- BROCK, R. Functional obstruction of the left ventricle. Guy's Hosp. Rep., 108: 126, 1959.
- Brachfeld, N. and Gorlin, R. Subaortic stenosis. A revised concept of the disease. *Medicine*, 38: 415, 1959.
- Brent, L. B., Aburane, A., Fisher, D. L., Moran, T. J. Myers, J. D. and Taylor, W. J. Familial muscular subaortic stenosis. An unrecognized form of "idiopathic heart disease" with clinical and autopsy observations. Circulation, 21: 167, 1960.
- Johnson, A. M. Functional infundibular stenosis; its differentiation from structural stenosis and its importance in atrial septal defect. Guy's Hosp. Rep., 108: 373, 1959.
- GORLIN, R. and GORLIN, S. G. Hydraulic formula for calculation of area of stenotic mitral valve, other cardiac valves, and central circulatory shunts. Am. Heart J., 41: 1, 1951.
- Ross, J., Jr., Braunwald, E. and Morrow, A. G. Transseptal left heart catheterization. A new diagnostic method. *Progr. Cardiovasc. Dis.*, 2: 315, 1960.
- BROCK, R. Control mechanisms in the outflow tract of the right ventricle in health and disease. Guy's Hosp. Rep., 104: 356, 1955.
- KIRKLIN, J. W., CONNOLLY, D. C., ELLIS, F. H., JR., BURCHELL, H. B., EDWARDS, J. E. and WOOD, E. H. Problems in the diagnosis and surgical treatment of pulmonic stenosis with intact ventricular septum. Circulation, 8: 849, 1953.
- CAMPBELL, M. and BROCK, R. The results of valvotomy for simple pulmonary stenosis. *Brit. Heart* J., 17: 229, 1955.
- HIMMELSTEIN, A., JAMESON, A. G., FISHMAN, A. P. and HUMPHREYS, G. H. II. Closed transventricular valvulotomy for pulmonic stenosis. Description of a new valvulotome and results based on pressures during operations. Surgery, 42: 121, 1957.
- ENGLE, M. B., HOLSWADE, G. R., GOLDBERG, H. P., LUKAS, D. S. and GLENN, F. Regression after open valvotomy of infundibular stenosis accompanying severe valvular pulmonic stenosis. *Circulation*, 17: 862, 1958.
- RODBARD, S. and REKATE, A. C. Direct evidence of supraventricular sphincter action as a mechanism of pulmonic stenosis. *Exper. Med. & Surg.*, 15: 317, 1957.
- HARRIS, P. Some variations in the shape of the pressure curve in the human right ventricle. Brit. Heart J., 17: 173, 1955.
- BASSETT, H. F. M. Pulmonary valvular stenosis. The infundibular factor in relation to valvotomy. *Thorax*, 13: 204, 1958.

- Teare, R. D. Assymetrical hypertrophy of the heart in young adults. Brit. Heart J., 20: 1, 1958.
- 20. Hollman, A., Goodwin, J. F., Teare, R. D. and Renwick, J. Clinical features of asymmetrical hypertrophy of the heart. A study of an affected family. *Brit. Heart J.*, 22: 449, 1960.
- 21. НЕСНТ, H. H. Anomalous atrioventricular excitation. Ann. New York Acad. Sc., 65: 826, 1957.
- 22. Gray, I. R. Paradoxical splitting of the second heart sound. *Brit. Heart J.*, 19: 303, 1957.
- Wood, P. Aortic stenosis. Am. J. Cardiol., 1: 553, 1958.
- COOPER, T., BRAUNWALD, E. and MORROW, A. G. Pulsus alternans in aortic stenosis. Hemodynamic observations in 50 patients studied by left heart catheterization. Circulation, 18: 64, 1958.
- MORROW, A. G., BRAUNWALD, E. and Ross, J., JR. Left heart catheterization: An appraisal of techniques and their applications in cardiovascular diagnosis. Arch. Int. Med., 105: 645, 1960.

The Effects of Intermittent Positive Pressure Breathing on the Intrapulmonary Distribution of Inspired Air*

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MECHANICAL aids to respiration have been widely used during anesthesia and to treat respiratory insufficiency in patients suffering from poliomyelitis [1,2], emphysema [3], chest injuries [4] and following thoracic surgery [5].

There have been a number of reports comparing the various aspects of pulmonary function before and after periods of intermittent positive pressure breathing [2,5]. Some investigators have described the pressure volume changes occurring during such periods of breathing [6], others the effect on the circulation [7], but none have described the effect on the intrapulmonary mixing of gases. The present study was therefore undertaken to compare the distribution of inspired gas during normal pressure breathing and during intermittent positive pressure breathing and voluntary hyperventilation at similar large tidal volumes in normal subjects and emphysematous patients.

METHODS

The distribution of inspired gas was studied by an open circuit nitrogen washout method in twelve normal subjects and in nine patients with obstructive emphysema; the same method was also used to estimate the functional residual capacity (FRC) in each instance [15–16]. All the patients had dyspnea and the physical and radiologic stigmas of emphysema with an obstructive pattern in the pulmonary function tests. (Table 1.)

The technic and apparatus used for the observations made during normal breathing and voluntary hyperventilation was similar to that described by Bouhuys, Hagstam, and Lundin [9]. Respiratory valves were arranged so that the subject inspired from a Douglas bag previously filled with oxygen and expired into a

Tissot spirometer as shown in Figure 1A. The air at the mouthpiece was continually sampled by a needle inserted as close to the mouth as possible and leading through a sampling tube to a nitrogen meter and vacuum pump. The concentration of nitrogen in the expired air could therefore be read on the meter dial and continuously recorded by a Sanborn direct writer as shown in Figure 2.

For the observations made during intermittent positive pressure breathing the expiratory valve of a Bird intermittent positive pressure breathing apparatus was connected by a very short length of brass tubing to the mouthpiece of the apparatus as shown in Figure 1B. Valve B was closed to disconnect the Douglas bag from the circuit. The patient inspired oxygen from the Bird intermittent positive pressure breathing apparatus and the expired air was sampled by the needle at the mouthpiece and collected and mixed in the Tissot spirometer as in the control experiments.

Before and between each experiment the entire apparatus was washed out with oxygen from the same cylinder, the unavoidable inert gas content of which was known and was in all cases below 0.9 per cent. The nitrogen meter was also calibrated before and between each experiment with oxygen mixtures in which the nitrogen concentration was known. Each subject rested for at least twenty minutes before and between each study, all of which were carried out in the sitting position. At least two studies were made for normal breathing, hyperventilation and intermittent positive pressure breathing to confirm that the results of the FRC obtained checked to within 5 per cent.

Normal breathing was first studied in each subject; normal breathing of atmospheric air was first established with valves A and B open to the atmosphere. (Fig. 1A.) At the end of a normal expiration the valves were closed so that inspiration was from an oxygen-filled Douglas bag and expiration was into the Tissot spirometer. After the start of oxygen breathing,

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TABLE I
RESULTS FOR NORMAL SUBJECTS

Normal	Type of	Tidal	Respira-	Functional Residual	Liters to Washout (each liter		Ve	entilato	ry Syste	emst		ber of N ₂ Mc	Breaths blecules	Pulmonar
Subjects and Age (yr.)	Breath- ing*	Volume BTPS (L).	Rate (per min.)	Capacity BTPS (L.)	of functional residual		Slo	west		Fas	test		ain in ings	Nitrogen Delay (%)
					capacity)	Fi	W_1	F ₂	W ₂	F ₃	W ₃	Ideal	Actual	
P. E., 35	NB	.65	11	2.740	10.2	.50	.944	.41	.854	.09	.636	7.6	12.0	58
	VH	1.50	10	2.740	8.2	.24	.890	.47	.695	.29	.588	3.3	4.5	36
	IPPB	1 20	10	2.980	10.0	.28	.933	.44	.739	.28	.659	6.1	6.7	10
F. E., 35	NB	.38	16	2.260	10.5			.96	.945	.03	.724	15.4	18.0	17
	VH	1.50	11	2.100	10.0					1.00	.691	3.2	3.2	0
	IPPB	1.07	16	2.000	9.8			.67	.868	.33	.676	4.9	6.1	25
F. R., 27	NB	.32	12	1.400	9.0			.68	.903	. 32	.750	6.6	8.3	26
	VH	.73	20	1.300	8.5					1.00	.746	3.9	3.9	0
	IPPB	.65	16	1.200	10.0			.42	.846	.58	.639	3.9	5.0	28
W. B., 22	NB	53	11	1.320	10.0	.24	946	.39	.728	.37	.752	4.6	7.4	61
	AH	1.57	8	1.450	8.0					1.0	.637	2.7	2.7	0
	IPPB	1.96	13	1 210	8.0					1.0	.643	2.8	2.8	0
F. G., 27	NB	.69	20	1.130	9.0					1.0	.792	5.0	5.0	0
	VH	1.72	6	1.000	11.4					1.0	.429	1.8	1.8	0
	IPPB	1.33	2	1.110	10.7					1.0	.653	2.8	2.8	0
R. J., 30	NB	.98	5	3.130	7.5					1.0	.822	5.6	5.6	0
	VH	1 44	7	3.010	7.4					1.0	.791	4.3	4.8	0
	IPPB	1.50	6	3.140	9.0			.69	.667	.31	.428	2.3	2.3	0
A. R., 35	NB	_70	10	1.900	14.3	.39	.951	.43	.783	.18	.647	5.2	10.5	102
	VH	1.49	8	1 710	8.6			.60	.813	.40	.452	2.3	3.9	70
	IPPB	1.57	10	1.630	13.0	.24	903	.43	.546	. 33	.466	2.6	3.8	46
P. T., 31	NB	.42	14	2_400	11.2	.54	966	.38	.901	.08	.707	8.8	18.2	107
	VH	1.38	10	2.200	6.3	. 33	853	.46	.676	.29	.525	3.2	4.1	28
	IPPB	1.24	9	2.300	8.9			44	.890	56	.630	3.4	5.5	62 37
L. L., 31	NB	.46	11	1.610	10.0			.70	.926	.30	.768	7.9	10.8	33
	VH	1.04	11	1.650	8.0			.67	.835		.566	3.6	5.2	26
C N: 22	IPPB NB	51	16	2.400	10.8	60	970	.66	.849	.34	.366	7.7	21.8	183
S. N., 22	VH	1.69	11	2 500	9.0	.60		.45	.871	.55	.560	3.0	4.7	57
	IPPB	1.60	11	2 600	11.0					1.00	.712	3.5	3.5	0
Z. R., 28	NB	.40	12	2 090	8.7					1.0	922	12.8	12.8	0
E. E., 20	VH	.72	12	2.050	8.0					1.0	.852	4.5	4.5	0
	IPPB	.65	17	1.850	9.8					1.0	.883	4.6	4.6	0
W. D., 22	NB	1.20	10	1.220	8.0					1.0	.691	3.2	3.2	0
	VH	1.40	13	1.210	7.0					1.0	.430	1.9	1.9	0
	IPPB	1.90	14	1 220	9.0					1.0	. 585	2.4	2.4	0
Mean values	NB	,60	12	1.970	9.9							7.5	11.1	48
	VH	1.34	11	1.910	8.4							3.2	3.7	17
	IPPB	1.31	12	1.911	10.0							3.6	4.2	16

^{*} NB indicates normal breathing; VH indicates voluntary hyperventilation; IPPB indicates intermittent positive pressure breathing.

† See text.

the end tidal nitrogen concentration fell progressively and these concentrations were recorded until the end tidal nitrogen concentration of the expired air had fallen to 2 per cent in the normal subjects, or for seven minutes in the emphysematous patients even though the end tidal nitrogen concentration was still more than 2 per cent; valves A and B were then opened to the atmosphere at the end of a normal expiration. The volume and temperature of the expired gas in the spirometer were recorded.

Intermittent positive pressure breathing was studied in the same patient by the arrangement with the Bird intermittent positive pressure breathing apparatus already described. From the volume of expired air collected in the Tissot spirometer and the number of breaths involved the tidal volume was calculated for the third part of each experiment. The rate was also noted

Voluntary hyperventilation was studied using the same apparatus and circuit as for normal breathing. The dial of a gasometer was visible to the subject so that with practice and a metronome he was able to ventilate at a tidal volume and rate similar to that employed during the intermittent positive pressure breathing experiment.

These three studies were carried out during (1) normal breathing, (2) intermittent positive pressure breathing, and (3) hyperventilation in all the normal subjects and all emphysematous patients except two who were unable to hyperventilate. The FRC and lung clearance indices were calculated, the nitrogen washout curves were graphically analyzed and the

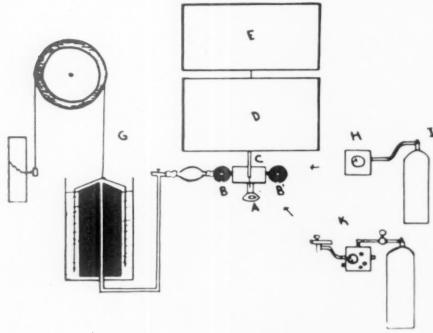


Fig. 1. The arrangement of the apparatus used for the study of the effects of intermittent positive pressure breathing. During normal breathing the patient breathes at mouthpiece (A) but when breathing with intermittent positive pressure breathing the valve K is inserted into the system. The other labelled parts are: B, three-way valve on the expiratory side; P, pneumotachograph; C, sample tubing and needle valve; D, nitrogen meter; E, recorder; B', three-way valve on the inspiratory side; I, oxygen supply and H, meter; K, intermittent positive pressure valve; and G, spirometer to collect the expired air.

pulmonary nitrogen clearance delay percentages calculated as follows:

The functional residual capacity (FRC) was calculated for each experiment by the method described by Darling [10] from the formula:

$$FRC = \frac{(V + D)(F_{sp} - F_{T}) - C}{F_{a_{0}} - F_{a_{n}}} - X$$

where V = volume of spirometer gas at blood temperature ambient pressure and saturated with water vapour.

D = known dead space of the spirometer circuit (560 cc.).

 F_{s_p} = percentage of nitrogen in the spirometer gas.

 F_T = percentage of nitrogen in the oxygen supply tank (below 0.9 per cent).

C = Correction for nitrogen excreted from the body during oxygen breathing = (body surface area × 96.5) 35 cc. [11].

X = dead space of the mouthpiece assembly between valve A and the teeth,
 (106 cc. for intermittent positive pressure breathing),

(52 cc. for normal pressure breathing).

The lung clearance index was described by Becklake [12] and is a measure of the over-all efficiency of

ventilation. It is the number of liters of ventilation required to wash out each liter of FRC down to 2 per cent end tidal nitrogen concentration. It is obtained by dividing the FRC in liters into the total volume in liters of the expired gas collected in the spirometer.

The pulmonary clearance delay percentage is an indication not of the over-all efficiency of ventilation but of the uniformity of ventilation in the different parts of the lungs. It was described by Fowler et al. [13] based on the fundamental equation of Darling et al. [14] who described the fall of mean expired nitrogen concentration during oxygen breathing in an evenly ventilated system.

$$F_{a_n} = F_{a_0} w^n \operatorname{Log} Fa_n = Fa_0 + n \operatorname{Log} w \dots (1)$$

where Fa_o = alveolar nitrogen concentration before oxygen breathing

 Fa_n = alveolar nitrogen concentration after n breaths of oxygen

$$w = \frac{VL}{V_L = V_T - V_D}$$
 is called the alveolar dilution factor

V_L = functional residual capacity

 V_T = tidal volume

V_D = anatomical dead space

Fowler [13] actually measured the mean expired nitrogen concentrations but in the present experi-

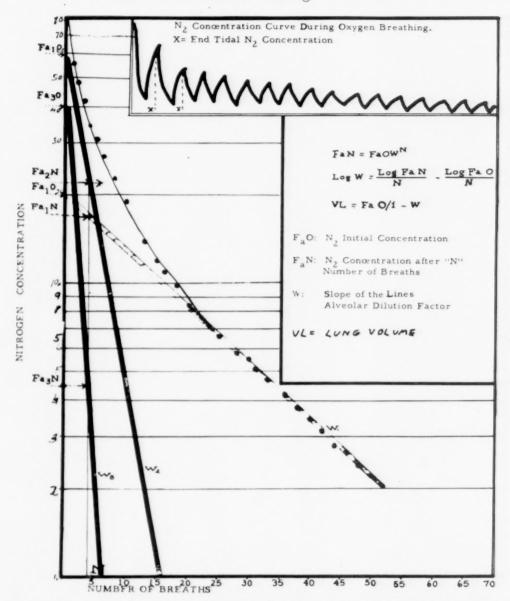


Fig. 2. Chart of semilogarithmic plot of the nitrogen concentration washout curve for a normal subject. The upper record enclosed in the box is of the breath-to-breath end tidal nitrogen concentration from which the washout curve is derived. The formula used for the value is seen in the lower rectangular box; a full discussion is found in the text.

ments the end tidal nitrogen concentrations were measured as in the simplification described and justified by Bouhuys [9]. The end tidal nitrogen concentration (F_a) for each expiration was measured from the nitrogen washout tracing and plotted on a semilogarithmic scale against the number of breaths (n) as in Figure 3. It will be seen from Equation 1 that the nitrogen concentration falls exponentially during the breathing of oxygen and appears as a straight line on the semilogarithmic plot when the ventilation is uniform. The alveolar dilution factor (w) is represented by the slope of the straight line, any departure from which expresses uneven ventilation. This is in fact found in many normal subjects and the

calculations given by Fowler [13] indicate that in these circumstances the over-all ventilation is the resultant of the sum of several parallel lung spaces which are ventilated at different rates represented by w_1, w_2, \ldots etc. These values w_1, w_2, \ldots etc. can be graphically determined by extrapolating the straight part of the curve towards zero and subtracting this line from the total curve. When the graph is resolved into two or more straight lines each represents a separate lung space itself evenly ventilated, the rate in each space being represented by an expression based on Equation 1. The values of Fa_1n , Fa_2n ... etc. and Fa_1o ... etc. and n, are read off from the graphs and the values of w_1, w_2, \ldots etc. readily calculated.

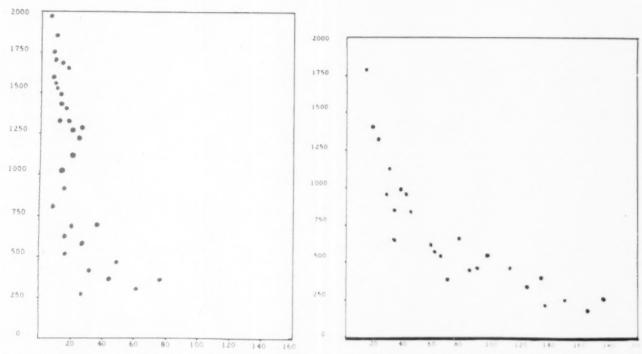


Fig. 3. Relation between tidal volume in milliter (ordinate) and number of breaths (abscissa) required to reach end tidal nitrogen concentration of 2 per cent in normal subjects (left) and in emphysematous patients (right).

The volume for each fraction is found from:

$$V_{L_1} = \frac{V_{L_1}}{1 - w_1}, V_{L_2} = \frac{F_{2_{20}}}{1 - w_2}, \cdots$$
 etc.

These formulas being derived from the mathematical expression for the sum of the terms of a decreasing geometric progression. $V_{L_1},\ V_{L_2},\ \dots$ etc. are theoretical numbers which represent the total amount of nitrogen washed out of each system; therefore, knowing the sum of all the fractions $V_{L_1},\ V_{L_2},\ \dots$ etc., the percentage volume $f_1,\ f_2,\ \dots$ etc. of each space can be calculated. That is:

$$f_1 = \frac{V_{L_1}}{V_{L_1} + V_{L_2} + V_{L_3}}, \qquad f_2 = \frac{V_{L_2}}{V_{L_1} + V_{L_2} + V_3}$$

Fowler [13] showed that the average number of breaths during which an average nitrogen molecule remains in a system, from which nitrogen is evenly or completely washed out, is $\frac{1}{1-w}$. In a system with several fractions with different washout rates or uneven ventilation the actual average number of breaths is

$$\frac{f_1}{1-w_1}+\frac{f_2}{1-w_2}\cdot\cdot\cdot\text{ etc.}$$

It is also possible to calculate what the ideal average number of breaths would be if the system had the same total V_L and $(V_T - V_D)$ as the several fractions but was evenly ventilated, the alveolar dilution factor

being W. In these circumstances this value of W can be calculated because it can be shown that:

$$\frac{1}{W} = \frac{f_1}{w_1} + \frac{f_2}{w_2} \cdot \cdot \cdot \text{ etc.}$$

The ideal average number of breaths is then $\frac{1}{1 - W}$

By comparing the actual and ideal average number of breaths an indication is obtained of the unevenness of ventilation. Even in normal subjects the actual number is usually larger than the ideal figure and this difference is expressed as the percentage of pulmonary nitrogen delay

which =
$$\frac{\text{(actual average number of breaths} - 1)}{\text{ideal average number of breaths}} \times 100$$

In normal subjects the percentage ranges from zero to 100 per cent according to Bouhuys [9].

RESULTS

These are summarized in Table 1 for the normal subjects and Table 11 for the emphysematous patients.

The functional residual capacity estimations during normal breathing, voluntary hyperventilation and intermittent positive pressure

TABLE II*
RESULTS FOR EMPHYSEMATOUS PATIENTS

Emphy- sematous	T	Tidal	Respira-	Functional Residual	Liters to Washout (each liter		Ve	entilato	ry Syste	ems		ber of	ge Num- Breaths olecules	Pul-
Patients and Age (yr.)	Type of Breath- ing	Volume BTPS (L.)	tory Rate (per min.)	Capacity BTPS (L.)	of functional residual		Slo	west		Fa	stest		ain in ngs	Mitroger Delay (%)
17.7			And the second s	(20.)	capacity)	F1	W ₁	F2	W ₂	F ₃	W ₃	Ideal	Actual	(707
S. CH., 49	NB	.40	22	2.84	16.4	.63	.938	.29	.923	.08	.804	21.7	56.9	162
	VH	.87	7	3.00	10.0			.75	.972	.25	.943	44.0	31.2	100
	IPPB	.99	9	3.06	12.4			.73	.923	.27	.772	8.3	10.8	30
F. G., 63	NB	.40	19	3.98	12.9	.73	.983	.23	.892	.08	.721	18.8	45.4	141
	VH	.53	17	3.90	13.6	.59	.977	.33	.854	.08	.620	9.3	28.3	202
	IPPB	.72	17	3.99	14.9	.46	.976	.39	.893	.15	.783	11.3	25.8	128
H. B., 52	NB	.46	20	3.46	18.6	.63	.992	.27	.949	.10	.881	31.0	87.9	183
	VH	.60	18	3.52	16.3	.35	.980	.48	.917	.17	.885	12.6	18.5	47
	IPPB	1.33	12	3.84	16.6			.73	.957	.57	.824	8.9	17.0	91
R. P., 70	NB	.50	24	3.80	15.0	.47	.992	.43	.918	.10	.694	14.6	50.3	244
	VH	.61	22	3.50	12.0	.52	.984	. 34	.843	.14	.702	8.9	36.0	304
	IPPB	1.20	9	3.00	14.0	.52	.968	. 30	.868	.18	.650	5.0	18.0	260
4. 1.	NB	.90	8	2.68	17.0	.60	.972	.28	.771	.12	.595	6.5	34.0	423
	VH	1.03	8	2.72	16.0	.71	964	.22	.663	.07	.246	7.9	20.8	163
	IPPB	1.43	5	2.82	13.0			.70	.921	.30	.653	5.7	9.8	71
I. J., 60	NB	.43	10	3.74	17.0	.49	.996	.40	.914	.09	.672	6.5	34.0	423
	VH	.65	10	3.20	15.0	.56	.997	.34	.838	.10	.604	7.7	19.4	152
	IPPB	.70	8	3.70	14.0	.55	.959	.34	.859	.11	.622	8.5	16.4	93
H. O., 66	NB	.82	8	3.00	17.0	.59	.971	.29	.770	.12	.594	6.3	33.7	403
	VH	1.00	8	3.60	12.0	.61	.950	.29	.691	.09	.307	7.9	20.8	163
	IPPB	1.80	5	3.20	16.1			.67	.953	. 33	.830	10.8	16.0	48
Ł. L., 64	NB	.39	29	4.00	16.0	.54	.979	.35	.879	.10	.706	7.5	29.0	288
	IPPB	.50	30	3.60	13.0			.84	.965	.16	.675	14.0	24.0	72
L. J., 71	NB	. 25	19.4	2.10	38.7	.44	.989	.38	.914	.18	.836	14.1	47.1	234
	IPPB	. 50	15	2.30	32.1	39	.982	.41	.895	.20	.809	10.9	27.1	148
Mean values	NB	.51	17.7	3.26	18.5							14.6	44.6	249
	VH	.76	13.0	3.39	13.6							8.0	24.9	182
	IPPB	1.02	12.2	3.28	16.2							9.2	18.4	100

* See footnotes to Table 1.

breathing did not show consistent changes in any one direction.

The over-all efficiency of ventilation, as expressed by the number of liters of oxygen to wash the nitrogen out of each liter of functional residual capacity (Becklake's lung clearance index), was not significantly different during normal breathing, voluntary hyperventilation and intermittent positive pressure breathing in the normal subjects; the mean values obtained were 9.9 L., 8.4 L. and 10 L., respectively. In the emphysematous patients there was a slight improvement, the mean decreasing from 18.5 L. for normal pressure breathing to 13.6 L. on voluntary hyperventilation and 16.2 L. on intermittent positive pressure breathing.

The number of breaths required to reach the end tidal nitrogen concentration of 2 per cent decreased in both the normal subjects and the emphysematous patients with an increase in tidal volume whether this was obtained by voluntary hyperventilation or intermittent positive pressure breathing. (Fig. 3.) Some of the

patients did not reach the 2 per cent level within seven minutes but the relationship was still evident and was to be expected from the definition of W (alveolar dilution factor).

In none of the normal subjects did graphic analysis of the semilogarithmic plot of the nitrogen washout indicate more than three ventilatory systems with different washout rates; four showed only one space, three showed two spaces and five showed three spaces. With the emphysematous patients there were always three or more spaces and the slowly ventilated compartments formed the major part of the total alveolar volume.

The calculations for the lung clearance delay were based on the three main systems only. In the five normal subjects in whom there was more than one system during normal breathing, and in all the emphysematous patients, an increase in tidal volume, whether achieved by voluntary hyperventilation or intermittent positive pressure breathing, resulted in an increase in the more rapidly ventilated spaces at the

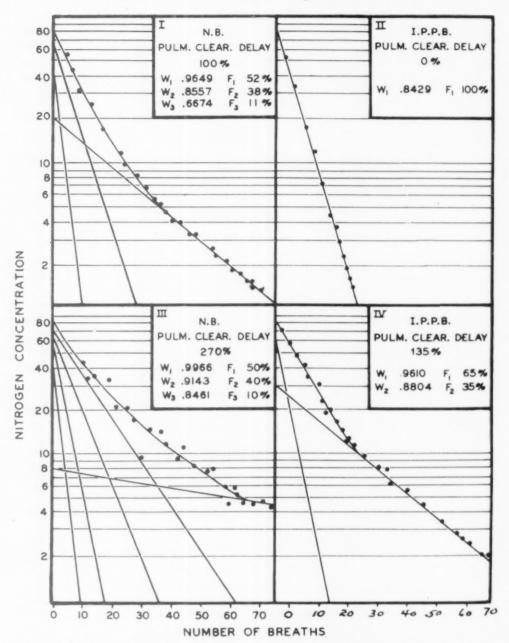


Fig. 4. Two graphs of the nitrogen washout curve in two emphysematous patients. The left is during normal breathing and the right is during intermittent positive pressure breathing. The slow washout curve, which is composed of several separate ventilatory spaces, is converted to a rapid washout curve and only one and two ventilatory spaces by intermittent positive pressure breathing. Note also that even the poorer ventilatory space is better ventilated than the moderately well ventilated space during normal breathing. This is related to the increase in tidal volume during pressure breathing. (See text for discussion.)

expense of the poorly ventilated spaces. In three of the four normal subjects in whom there was only one ventilatory space on normal breathing, both voluntary hyperventilation and intermittent positive pressure breathing an additional system appeared; even so the pulmonary clearance delay remained unaltered at zero indicating that the lungs were still evenly ventilated.

In the normal subjects during normal breathing the ideal and the actual numbers of breaths, the nitrogen molecules remained in the lungs. They decreased during voluntary hyperventilation and intermittent positive pressure breathing; the change was reflected by the fall in the lung clearance delay from a mean of 48 per cent to 17 per cent and 16 per cent, respectively.

In the emphysematous patients the mean value of the ideal number of breaths (14.1) was greater than in the normal subjects, so that even if ventilation had been uniform the low effective alevolar ventilation per breath allowed the nitrogen molecules to remain longer in the lungs. The actual number of breaths also was higher (mean 44.6). The mean value of the lung clearance delay in the emphysematous patients decreased more than 100 per cent. During normal breathing this value was 249 per cent and it was improved during intermittent positive pressure breathing to a mean of 100 per cent. In the patients who were able to hyperventilate the improvement was similar and to a mean of 159 per cent. Two of the patients were unable to increase their tidal volume voluntarily. (Fig. 4.)

COMMENTS

The variation in the results of the estimations of functional residual capacity during intermittent positive pressure breathing and voluntary hyperventilation are similar to those found by Bouhuys [9] during the experiments with hyperventilation. The FRC increases during continuous positive pressure breathing [14] and may also increase following a period of intermittent positive pressure breathing [15]. The present experimental method is based on the amount of nitrogen in the lungs at the end expiratory level just before the first inspiration at positive pressure; any observed change cannot be directly due to the intermittent positive pressure breathing or voluntary hyperventilation except that these methods wash out nitrogen from the lungs more completely and may therefore give higher FRC results, particularly in patients with uneven ventilation on normal breathing. No such consistent changes were found and it seems probable that the variation in the results is due to changes in the resting expiratory level at the start of oxygen breathing under the various circumstances. Although such variations in determining the resting expiratory level at which to turn the patient from air to oxygen breathing affect the determinations of functional residual capacity and, therefore, to some extent the results of the Becklake's index, Bates [17] has shown that the level of the end expiratory volume does not affect the distribution of the inspired air.

The over-all efficiency of ventilation was not significantly altered in normal subjects either by voluntary hyperventilation or intermittent positive pressure breathing; in the emphysematous subjects there was an improvement when the tidal volume was increased. The improvement was greater with voluntary hyperventilation than with intermittent positive pressure breathing and is therefore related to the more effective alveolar ventilation obtained by the increase in tidal volume and not to the pressure at which the oxygen was breathed.

In both the normal subjects and emphysematous patients the evenness of the distribution of ventilation was similarly improved by both voluntary hyperventilation and intermittent positive pressure breathing. Again the change is related to the increase in tidal volume and not to the pressure of administration. Some of the emphysematous patients were unable to increase their tidal volume voluntarily and in these subjects intermittent positive pressure breathing did achieve an increase in tidal volume and therefore the improvement in the evenness of ventilation.

There is some theoretical and experimental evidence [17] that, where ventilation is initially uneven, an increase in the flow rate, as might be expected from the use of intermittent positive pressure breathing, will so alter the time constants of the airways that there will be an increase in the unevenness and decrease in the over-all efficiency of ventilation, but emphysematous patients will not tolerate higher flow rates. It is probable that the failure of intermittent positive pressure observed by Gaensler and Lindgren [19] may be accounted for by these factors.

It is concluded, therefore, that the beneficial effects of intermittent positive pressure breathing on the ventilation of the lungs are due to the increase in tidal volume obtained. The method is of particular value in patients who are unable to increase and maintain an increase in their tidal volume voluntarily. In practice there is not likely to be any impairment of efficiency or decrease in the evenness of ventilation by any flow rate tolerable to the patient.

SUMMARY

The effect of intermittent positive pressure on the intrapulmonary mixing of inspired gas was studied in twelve normal subjects and nine emphysematous patients by the method of nitrogen washout.

The over-all efficiency of ventilation, as

measured by Becklake's lung clearance index, was not improved by intermittent positive pressure breathing in the normal subjects and only to a small degree in the emphysematous patients.

The evenness of ventilation as measured by Fowler's percentage of lung clearance delay was improved in both the normal subjects and to an even greater degree in the emphysematous

patients.

In all the normal subjects and in those emphysematous patients who could do so, a voluntary increase in tidal volume comparable to that produced by intermittent positive pressure breathing effected the changes in the over-all efficiency of ventilation. It is concluded that the beneficial effects of intermittent positive pressure breathing on the intrapulmonary mixing of gases are due to the increase in tidal volume obtained, and are of particular value in those patients who are unable to maintain an effective tidal volume or to increase it.

There was no evidence that the flow rates produced in practice with intermittent positive pressure breathing were high enough to alter the time constants of the airways and so accentuate any initial unevenness of ventilation.

REFERENCES

 Lassen, H. C. A. The 1952 epidemic of poliomyelitis in Copenhagen. *Lancet*, 1: 37, 1953.

 SYRREL, W. E. and Tomashefski, J. F. Results in flight testing of Sam-Portable respirator on poliomyelitic patients. J. Aviation Med., 25: 245, 1954.

 MOTLEY, H. L. and TOMASHAFSKI, J. F. Treatment of chronic pulmonary disease with intermittent positive pressure breathing. *Arch. Indus. Hyg.*, 5:1, 1952.

 Boyle, A. K., Gallie, J. R. and Murray, D. B. Crush injury of the chest, a report of two cases.

Anaesthesia, 12: 4, 1957.

 BJORK, V. O. and ENGSTROM, C. G. The treatment of ventilatory insufficiency after pulmonary resection with tracheostomy and prolonged artificial ventilation. J. Thoracic Surg., 30: 356, 1955.

- SEGAL, M. S., SALOMON, A., DULFANO, M. J. and HERCHFUS, J. A. Intermittent positive pressure breathing. Its use in the inspiratory phase of respiration. New England J. Med., 25: 225, 1954.
- OPIE, H. L., SPALDING, J. M. K. and STOTT, F. D. Mechanical properties of the chest during intermittent positive pressure breathing. *Lancet*, 14: 545, 1959.
- Feinsilver, O. Circulatory changes associated with inspiratory positive pressure breathing. *Dis. Chest*, 34: 187, 1958.
- BOUHUYS, A., HAGSTAM, K. E. and LUNDIN, G. Efficiency of pulmonary ventilation during rest and light exercise. A study of alveolar nitrogen washout curves in normal subjects. *Acta physiol.* scandinav., 35: 121, 289, 1955–56.

 DARLING, R. C., COURNAND, A. and RICHARDS, B. W., Jr. Studies on intrapulmonary mixture of gasses. III. An open circuit method for measuring residual air. J. Clin. Invest., 19: 609, 1940.

COURNAND, A., YARMUSH, I. G. and RILEY, R. L.
The influence of body size on gaseous nitrogen
elimination during high oxygen breathing.
Proc. Soc. Exp. Biol. & Med., 48: 280, 1941.

 BECKLAKE, R. M. A new index of the intrapulmonary mixture of inspired air. *Thorax*, 7: 111, 1952.

 FOWLER, W. S., CORNISH, E. R., JR. and SEYMOUR, S. K. Lung function studies—analysis of alveolar ventilation by pulmonary nitrogen clearance curves. J. Clin. Invest., 31: 40, 1952.

 DARLING, R. C., COURNAND, A. and RICHARDS, D. W. Intrapulmonary mixing of gases. J. Clin. Invest.,

23: 55, 1944.

- RAHN, H., OTIS, A. B., CHADWICK, L. W. and FENN, W. O. The pressure volume diagram of the thorax and lung. Am. J. Physiol., 146: 161, 1946.
- MOTLEY, H. L. and LANG, L. P. Intermittent positive pressure breathing combined with nebulization in pulmonary disease. Am. J. Med., 5: 853, 1948.
- BATES, D. V., FOWLER, W. S., FORSTER, R. E. and VAN LINGEM, B. Uniformity of ventilation at different lung volumes. J. Appl. Physiol., 10: 598, 1959.
- OTIS, A. B., McKerrow, B. C., Bartlett, R. A., McIlroy, J., Selverstone, N. J. and Radford, E. P. Mechanical factors in distribution of pulmonary ventilation. J. Appl. Physiol., 8: 427, 1056
- GAENSLER, E. A. and LINDGREN, I. The mechanics of breathing. Progr. Cardiov. Dis., 1: 397, 1959.

Pulmonary Function in the Hamman-Rich Syndrome*

The Abnormalities of Ventilation, Blood Gases and Diffusion at Rest and on Exercise

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THE clinical syndrome due to diffuse interstitial pulmonary fibrosis described by Hamman and Rich [1,2] is characterized by dyspnea and cyanosis, in some cases on exercise only, but in the more severe stages, even at rest. Other manifestations of this syndrome such as resting hyperventilation, cough and clubbing of the fingers are common. A pathologic disorder, the generalized fibrous thickening of alveolar septums is characteristic, and accounts for the difficulty in oxygen transfer. In many cases cystic or emphysematous spaces also develop in the lungs giving a honeycomb appearance [3,4]. The original patients who were reported on by Hamman and Rich all went rapidly downhill to an early death, but if this course is a requisite for diagnosis the condition cannot be diagnosed during life. Many patients are now included in this group despite a more prolonged course [3,5], provided they fulfill the clinical and pathologic criteria.

Rubin and Lubliner [3] have recently made a detailed study of this condition and Read [5] has reviewed it from the point of view of etiology, but a detailed study of pulmonary function has not been reported. Reduced lung volumes, normal or reduced maximum breathing capacity (MBC), and polycythemia have been noted by many observers in case reports. In addition, Silverman and Talbot [6] found a raised pulmonary artery pressure and arterial oxygen unsaturation in one case; and Merrill, Callaway and Meneely [7] reported on a patient with arterial oxygen unsaturation. Two other patients illustrate well the functional improvement

which may accompany successful corticosteroid therapy. Fleishman et al. [8] reported on a patient in whom the arterial oxygen saturation was followed up over a period; it was normal when the steroid dosage was adequate but fell when dosage was reduced and the patient was in a clinical relapse. This patient also showed a reduced pulmonary compliance. In the case of Read and Holland [9], the administration of cortisone resulted in restoration of the severely reduced lung volumes and MBC to normal, the resulting arterial oxygen unsaturation was relieved and the diffusing capacity doubled.

Pulmonary function has been studied in several series of patients who had pulmonary fibroses and granulomatoses but many of these have included no specifically identified case of diffuse fibrosis of the Hamman-Rich type [10–14]. The one patient of Cugell et al. [15] had a lowered diffusing capacity for carbon monoxide (Dco) on exercise and Bates [16] found exercise Dco lowered in three cases. Ogilvie et al. [17] found a lowered breathholding Dco at rest.

Recently Holland [18] has reported that the physiologic dead space is greatly increased in this condition, and has drawn attention both to the disabling effect of this and to its effect on the validity of some of the tests for diffusing capacity. The full results of the tests have not yet been presented. In the present paper the lung volume, blood gas, ventilation and diffusion studies in five cases of the Hamman-Rich syndrome are reported, particular attention

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being paid to the diffusing capacity at rest and on exercise.

MATERIAL AND METHODS

These have been largely described elsewhere [19] but will be outlined briefly. Lung volumes were determined on a 6 L. Knipping spirometer (C. F. Palmer), the functional residual capacity (FRC) being determined by the closed circuit helium technic, a modification of the method of McMichael [20]. Maximum breathing capacity was determined over twelve seconds breathing on the closed circuit. The values for lung volume and MBC have been compared with values predicted for the patient by the formulas of Needham et al. [21].

Ventilation, blood gas and diffusion studies were made at rest and on exercise. For the studies at rest the patient was propped up on a cardiac bed at an angle of 70 to 80 degrees; for studies on exercise a stationary ergometer bicycle was used. At any level of exercise, five minutes was allowed for a steady state to be reached. Diffusing capacity was determined by the steady state, physiological dead space method of Filley, MacIntosh and Wright [22]. A mixture of about 0.05 per cent carbon monoxide in air was breathed, the expired air being collected in a Douglas bag. Two to three minutes was allowed for equilibration of the inspired mixture with lung air at rest, and one to two minutes was allowed on exercise. Analysis of inspired and expired air for carbon monoxide was performed on an infra-red CO analyser (Model SCL, the Infra-Red Development Co., Welwyn Garden City, Hertfordshire, United Kingdom) setting against a standard mixture of 0.04 per cent CO in nitrogen supplied by the manufacturers. The linearity of the meter had been confirmed by circulating a CO mixture through the analyser and a spirometer and using the spirometer to add measured amounts of CO-free air to the system. All readings were within 1 per cent of the reading expected from the initial reading and the known dilution. Expired air was passed through ascarite to remove carbon dioxide before entering the analyser; and the CO reading corrected later to allow for this change in volume. Arterial blood was withdrawn from an indwelling needle evenly throughout the collection of gas. The pH of whole arterial blood was measured by a specially calibrated Cambridge pH meter using an anaerobic cell and a Stadie microelectrode. It was measured at the temperature of running water and corrected to 37°c. by the formula of Rosenthal [23]. Blood gases were estimated by the method of Van Slyke and Neill [24] with appropriate allowance for physically dissolved oxygen in the calculation of oxygen saturation. Expired air was analysed on the Haldane apparatus, duplicate values being required to check to within 0.02 per cent. Arterial CO2 tension was read from the nomogram of Singer and Hastings [25].

Studies at rest were usually performed in duplicate

and in the results only the mean is shown. On exercise, duplicate determinations were sometimes omitted and two or more levels of exercise studied.

Correction for back tension of CO due to the presence of carboxyhemoglobin in the blood was made in only one case. On theoretical grounds this was not regarded as an important source of error; this was confirmed on the one occasion the correction was applied and the reasons for this are discussed later.

Fractional CO uptake (CO_F) was calculated as (CO fraction in inspired gas – CO fraction in expired gas)/CO fraction in inspired gas. No correction was made for the ventilation of the mouthpiece.

Values of diffusing capacity and oxygen consumption are expressed at standard temperature and pressure dry (STPD); ventilation, alveolar ventilation, tidal volume and dead space are expressed at body temperature and ambient pressure saturated with water vapour (BTPS); and lung volumes and maximum breathing capacity are expressed at ambient temperature and pressure saturated with water vapour (ATPS).

Values for ventilation are given uncorrected for dead space of the mouthpiece and valve system. For a comparison with normal, they were compared with the value predicted for that consumption of oxygen using the formula obtained in this laboratory with the same or a similar mouthpiece and valve system used [19]. A similar comparison was made for alveolar ventilation. The relevant equations are:

$$\dot{\mathbf{V}}_{E} = 26.3\dot{\mathbf{V}}_{O_{2}} + 0.39$$

$$\dot{\mathbf{V}}_{A} = 24.9\dot{\mathbf{V}}_{O_{2}} - 1.63$$

The symbols used are the conventional ones [26,27].

Dyspnea on exercise was graded on the basis of the patient's apparent state at the end of exercise. The following four grades were used: mild, moderate, severe and maximal. Gradings were made by two observers and rarely differed by more than half a grade. Breathing reserve was calculated as the percentage of the MBC unused on exercise [10].

The Subjects of the Study. All the patients studied had the characteristic history, physical findings and radiologic appearance of the Hamman-Rich syndrome. There was no significant history of industrial exposure and the results of routine tests for other conditions were negative. In two cases (D. C. and W. S.) the diagnosis was confined by thoracotomy lung biopsy; in two other cases (W. J. and S. K.) autopsy findings were available as well; and in the remaining case (R. F.) the diagnosis rested on typical clinical and radiologic findings and a biopsy specimen of a scalene node that had negative results. Disability was severe in two cases (W. J. and W. S.), moderate in two cases (D. C. and S. K.), and mild in one case (R. F.). In all cases there was clubbing of the fingers and fine rales over at least the lung bases.

All were studied before the institution of any therapy. W. J. was studied two months later while on

TABLE I
PHYSICAL DATA, LUNG VOLUMES, AND MAXIMUM BREATHING CAPACITY OF THE PATIENTS STUDIED

	Age			Body Sur-			l Lung pacity		sidual lume	Residual Volume/	Vital	Capacity	Maximum ing Cap	
Patient	(yr.) and Sex	Height (in.)	Weight (lb.)	face Area (sq. M.)	Test	L.	% Pre- dicted	L.	% Pre- dicted	Total Lung Capacity (%)	L.	% Pre- dicted	L./min.	% Pre- dicted
W. J.	54, M	68	132	1.71	A	3.41	54 48	1.03	39 31	30.0 27.0	2.13	58 52	133 108	129 104
D. C.	32, M	71	121	1.72	A B	5.05	70 72	1.19	48 60	23.6 29.6	3.89	81 77	97 97	76 78
S. K.	17, F	60	88	1.31		1.88	46	0.67	74	35.6	1.11	44	40	40
R. F.	61, M	66	121	1.60		2.74	47	0.74	28	27.0	1.85	59	75	82
W.S.	57, M	65	153	1.76	A	4.22	77	1.00	45	23.7	3.24	100	85	83
					В	4.62	85	1.37	63	29.7	3.30	102	88	86

steroid therapy; D. C. fifteen months later, after a course of steroids; W. S. four months later, also after a course of steroids. In no case did the therapy make any apparent difference to the clinical condition. In the results the first series of tests is referred to as "A" and the second as "B."

RESULTS

The results in detail are shown in Tables 1 and 11 and in Figures 1, 2 and 3. In addition the basic physical data are shown in Table 1. The cases

did not form a homogeneous group as regards severity and so no attempt was made to calculate mean values for the series.

Lung Volumes and MBC. Lung volumes were reduced to varying extent, no subdivision being particularly affected. The MBC varied greatly, sometimes being higher than the predicted value and in one case (S. K.) considerably reduced. (Table 1.)

Physiological Dead Space. This was increased in

Table II
VENTILATION, BLOOD GASES AND DIFFUSING CAPACITY AT REST AND ON EXERCISE

			Ventila	ation	Alvee Ventila				Arterial	Blood		Frac-		Diffusing
Patient	Test	O ₂ Consumption (ml./min.)	L./min.	% Pre- dicted	L./min.	% Pre- dicted	Respira- tory Ex- change Ratio	O2 Satura- tion of Haemo- globin (%)	Packed Cell Volume (%)	рН	CO ₂ Tension (mm. Hg)	tional CO Up- take (%)	FA _{co} FI _{co}	Capacity for CO (ml./min., mm. Hg)
W. J.	A	288†	17.73	220	5.95	106	0.91	94.1	0.43	7.43	38.0	19	0.45	8.6
	В	285*	15.98	202	4.92	90	0.75	95.6	0.40	7.43	36.6	17	0.48	6.4
		593	34.22	214	11.60	88	0.83	77.1	0.41	7.40	36.5	13	0.64	8.0
D. C.	Α	252*	12.55	180	8.00	174	1.02	97.0	0.40	7.53	27.6	26	0.59	6.4
		827	43.84	198	22.71	120	1.02	89.3	0.44	7.43	32.1	15	0.71	10.9
	В	212	12.33	207	5.92	162	1.03	96.4	0.43	7.49	32.4	22	0.54	5.9
		221	15.18	245	5.85	151	1.15		0.43	7.52	29.6	21	0.57	6.5
		603	33.86	208	15.16	113	1.03	92.4	0.45	7.42	35.7	16	0.64	9.9
		784	47.76	227	20.43	114	1.00	84.6	0.46	7.42	33.7	14	0.68	11.2
S. K.		187*	7.72	146	3.19	105	0.80	93.2	0.36	7.42	41.0	25	0.37	6.0
		552	25.03	168	11.44	94	0.97	90.0	0.40	7.38	41.1	18	0.61	7.9
R. F.		254*	10.24	145	4.42	94	0.85	97.3	0.47	7.42	43.0	26	0.41	7.4
		506*	20.77	151	9.58	88	0.94	90.7	0.48	7.40	43.6	21	0.54	9.6
W. S.	A	212*	10.89	183	4.94	136	0.86	85.6	0.52	7.47	32.5	16	0.64	4.1
		443	27.16	226	11.56	123	0.93	83.1	0.52	7.47	31.1	13	0.71	5.7
		598	55.92	347	21.81	164	1.20	76.3	0.52	7.45	29.0	9	0.75	8.1
	В	234	13.29	203	5.24	125	0.89	88.7	0.52	7.44	35.0	15	0.63	3.6
	-	232	12.52	193	5.02	121	0.85	88.1	0.52	7.47	34.6	16	0.61	3.7
		505	35.60	260	12.71	116	1.03	70.6	0.52	7.42	36.1	11	0.69	6.6
		575	47.43	306	15.89	125	1.10	74.2	0.53	7.42	35.0	9	0.72	7.1

^{*} Mean of duplicate determinations at this level of exercise.

[†] Mean of three determinations

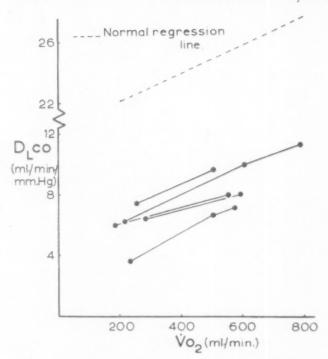


Fig. 1. Diffusing capacity in five patients with the Hamman-Rich syndrome and its rise with exercise. In this and the subsequent figures only one study is shown for each patient, and the study chosen is the same for each figure. The regression lines are those found previously in this laboratory [19].

all the cases as was reported previously by one of us [18]. On exercise this increase was also marked, the dead space reaching the value of 1,000 ml. in one case (W. S.). The increase in dead space was less in two cases (S. K. and R. F.) than in the other cases.

Diffusing Capacity. This was greatly reduced in all patients at rest and on exercise. (Table II and Fig. 1.) In any patient, Doo rose on exercise, the rise being approximately parallel to the regression line for normal subjects previously found in this laboratory [19]. None of the patients was able to do more than very light exer-

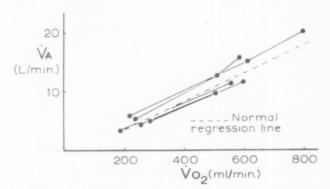


Fig. 3. Alveolar ventilation in five patients with the Hamman-Rich syndrome and its rise with exercise.

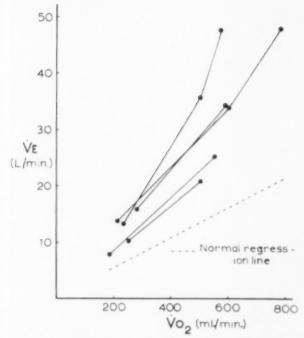


Fig. 2. Ventilation in five patients with the Hamman-Rich syndrome and its rise with exercise.

cise and thus the values obtained for Dco are maximal or near maximal for them.

Fractional CO Uptake (CO_F). This was below 30 per cent in all cases and was especially low in one case (W. S.) whose Dco was the most severly reduced.

Alveolar CO Tension. This was high in relation to inspired CO tension in all cases. In normal subjects in this laboratory, the ratio $P_{\rm Aco}/P_{\rm I_{co}}$ was usually below 0.25.

Arterial Oxygen Saturation. This was normal or only a little reduced at rest in all except one case (W. S.) whose saturation was below 90 per cent. On exercise, arterial oxygen saturation fell in all cases.

Arterial pH and CO₂ Tension. This varied from subject to subject and showed no definite pattern. On exercise pH always fell, but CO₂ tension remained much the same as at rest.

The Packed Cell Volume (PCV). This was raised in only one case (W. S.). He was the only one in the series to have chronic resting arterial oxygen unsaturation.

Ventilation and Alveolar Ventilation. Hyperventilation was present in all cases at rest and persisted or became more marked on exercise. (Table II and Fig. 2.) Alveolar ventilation was approximately as predicted in most cases. (Table II and Fig. 3.) However, two patients (D. C. and W. S.), showed high resting alveolar ventilations.

Table III

EFFECT OF BREATHING 100 PER CENT OXYGEN IN ONE
PATIENT (W. S.) AT REST

Measurement	Breath-		thing % O ₂
		1	2
Ventilation (L./min.)	12.52	10.26	9.93
Alveolar ventilation (L./min./sq. M.)	2.81 7.47	2.04	7.43
Arterial pH	34.6	7.43	37.3
Arterial O2 saturation (%)	88.1		97.9

To get more information on this, a comparison was made of their arterial blood gas figures before and after connection to the respiratory valves. In one case (D. C.) before connection the arterial pH, CO2 content, and CO2 tension were 7.45, 21.6 mM/L., and 36.5 mm. Hg, respectively. After connection in the first estimation of Dco the corresponding figures were 7.49, 20.8 and 32.4, and in the second estimation they were 7.52, 20 and 29.6. In one case (W. S.) the results were 7.44, 19.3 mM/L., and 34.2 mm. Hg before connection, and 7.44, 19.7 and 35 after connection. Thus in D. C. the raised alveolar ventilation was largely caused by the discomfort of the valves but in W. S. this was not so. This was also suggested by the high and variable respiratory exchange ratio in D. C. and the more normal and stable ratio of W. S.

The effect of breathing 100 per cent oxygen on the resting alveolar hyperventilation in W. S. was investigated. A period of twenty minutes was allowed for equilibrium before collections were begun. Oxygen consumption could not be measured but CO₂ output and alveolar ventilation were estimated. Table III shows the changes brought about by breathing 100 per cent oxygen: the haemoglobin became fully saturated, a fall in total ventilation occurred with alveolar ventilation dropping to normal, and there was a decrease in the alkalosis in the arterial blood.

The degree to which anoxemia on exercise was acting as a stimulus to breathing was investigated in two cases (D. C. and W. S.). After estimation of the diffusing capacity both patients continued to exercise at the same rate. Inspired gas was changed to 100 per cent oxygen and collection of expired air and arterial blood was begun at once. No attempt was made to achieve a steady state as the patients were not fit for prolonged exercise. The results are shown in

Table IV

EFFECT OF BREATHING 100 PER CENT OXYGEN IN TWO
PATIENTS (D. C. AND W. S.) ON EXERCISE

Measurement	D	. C	W.	S.
Measurement	Air	100 % O ₂	Air	100 % O ₂
Ventilation (L/min)	47.76	32.66	47.43	32.17
(L./min.) Ventilation of MBC				
(%) Dyspnea at end of	49.0	34 0	54.0	37.0
period	Maximal	Moderate	Very severe	Mild to moderate
Arterial pH	7.42	7.40	7.42	7.34
(mm. Hg)	33.7	35.9	35.0	41.2

Table IV. Breathing 100 per cent oxygen resulted in a fall in total ventilation and a rise in blood CO₂ and acidity, thus indicating that some of the stimulus to breathing was due to hypoxia.

COMMENTS

Lung Volumes and Maximum Breathing Capacity. Reduction in lung volume, with all subdivisions affected to a greater or lesser extent, has been the usual finding in "pulmonary fibrosis" [10]. On the basis of morbid anatomical changes it appears that two factors operate: first, the restriction of lung volumes and movements by the fibrotic process, and secondly, replacement of alveolar space by fibrous tissue. The relatively normal MBC indicates that there is minimal airway obstruction. At the time of testing, three patients (W. J., D. C. and W. S.) were known to have fine diffuse emphysema. Only one patient (S. K.) was known not to have it. Yet she had the lowest MBC of the series and the highest ratio RV/TLC, the rise in this ratio being due to disproportionate reduction of TLC. Three cases (S. K., R. F. and W. S.) had normal intrapulmonary mixing of helium and two (W. J. and D. C.) had only a slight defect [28]. These findings show the inability of the spirometric tests to diagnose fine diffuse emphysema. They are in accordance with the findings of Gilson and Hugh-Jones [29] who found that patients with coalworkers' pneumonokoniosis, a disease attended by much focal emphysema, had relatively little change in the RV/TLC ratio, in MBC, or in mixing, when compared with patients with vesicular emphysema.

The Diffusing Capacity for CO. This was measured by the method of Filley et al. [22] in this series. In this steady state method, arterial

CO₂ tension is taken as "alveolar" CO₂ tension and a value for the dead space/tidal volume ratio calculated from the Bohr equation:

$$\frac{V_{D}}{V_{T}} = \frac{P_{A_{CO_{2}}} - P_{E_{CO_{2}}}}{P_{A_{CO_{2}}} - P_{I_{CO_{2}}}}$$

In practice, when air is breathed, inspired CO₂ tension can be taken as zero and the equation simplified. This value for dead space/tidal volume ratio is used again in the rearranged Bohr equation to obtain a value for alveolar CO tension:

$$P_{Aco} = \frac{P_{Eco} - \frac{V_D}{V_T} P_{Ico}}{1 - \frac{V_D}{V_T}}$$

Thus the Filley method has a different basis from the method of Bates et al. [30] for Dco determination. This latter method, determining alveolar CO either by end-tidal sampling or by assumption of a dead space, is likely to underestimate Dco in the Hamman-Rich syndrome due to the high physiological dead space present [18].

The validity of the Filley method must also be considered. Forster, Fowler and Bates [31] showed that the validity of a steady state method depended on the uniformity throughout the lungs of the ratio:

$$\frac{\text{diffusing capacity of an alveolus}}{\text{ventilation of that alveolus}} \quad \frac{(D_L)}{(\dot{V}_A)}$$

Any departure from this results in an underestimation of the diffusing capacity. The Filley method may be examined from this point of view. Consider first a lung containing a number of alveoli in which the ratio D_L/V_A is the same; these constitute one diffusing phase. Furthermore, let the ventilation/perfusion ratio be the same in all these alveoli so that they all have the same CO₂ tension. Let this lung also contain a number of alveoli which are ventilated but neither perfused nor in contact with static blood; these have zero diffusing capacity and so there is non-uniformity of the ratio DL/VA (VA in this instance refers to anatomic alveolar ventilation). In any end-tidal sampling method of estimating alveolar CO tension, the unchanged gas from these unperfused regions with a high content of CO would be mixed with the gas from the other regions; the CO tension of the mixed gas would be higher than that of the

gas from the diffusing region; alveolar CO tension would be overestimated leading to a low value for diffusing capacity which would be false. On the other hand, in the Filley calculation, the unchanged air from the unperfused regions causes a lowering of expired CO2 tension, and it can be seen from inspection of the Bohr equation that the ratio VD/VT is increased. The unperfused alveoli appear as an increase in the physiological dead space, and the alveolar CO tension found is that in the perfused alveoli. The true value for Dco is found and to this extent the Filley method avoids errors due to non-uniformity. The high physiological dead space found in the Hamman-Rich syndrome suggests that this form of non-uniformity is present and for this reason the Filley method is a good one for use in this condition.

However, if the non-uniformity in the ratio D_L/\dot{V}_A is due solely to differences in the thickness of alveolar walls, the Filley method will be just as much in error as the alveolar sampling method. This is because of the very high diffusibility of CO_2 : varying thickness of alveolar septums does not, *per se*, result in any non-uniformity of physiological alveolar ventilation in the lung, whereas it does cause variations in diffusing capacity for oxygen or carbon monoxide. As the changes in the Hamman-Rich syndrome are not necessarily uniform [3,32], this form of non-uniformity may well be present.

The great difficulty is in assessing the effect of variations in the ratio of D_L/\dot{V}_A in different alveoli when the ratio:

$$\frac{\text{blood flow}}{\text{alveolar ventilation}} \frac{(\dot{Q}_C)}{(\dot{V}_A)}$$

also varies between anatomic alveoli. In the extreme case, where some alveoli have no perfusion, the Filley method gives the correct result as described. In less extreme cases the problem is whether or not the reduction in diffusing capacity in any given alveolus is related to reduction in blood flow so that the ratio

is the same as for other alveoli. If so, uniformity has been maintained, but it is difficult even to hazard a guess at these relations in a given case. The impression is that the Filley method is liable to error due to the non-uniformity but less so than the end-tidal sampling method.

Against the tendency for the variations in thickness in the alveolar walls to cause underestimation of Dco must be set the tendency for the use of physiological dead space to overestimate Dco [18,33]. The net effect is likely to be an underestimation of Dco [33]. Examination of the present series of results does not indicate serious error in the Filley technic in this condition.

No allowance was made for back tension of CO in equilibrium with carboxyhemoglobin in the pulmonary capillaries. Three factors reduce its importance in this condition. First, the total uptake of CO is less because of the impaired diffusion; thus there is less build-up of COHb in the blood in the course of a test. Secondly, also due directly to the diffusion defect, the alveolar CO tension is higher and thus any back tension existing is relatively less important. Thirdly, the lowered oxygen tension in the pulmonary capillaries will reduce the back tension from any given COHb concentration. The presence of reduced haemoglobin also lessens back tension [27,31,34]. To check this, the correction was estimated in one case (W. S.). He had smoked twenty-five cigarettes the day before and five in the morning before testing. The COHb was estimated by the method of Roughton and Root [35] and hence back tension by the method of Linderholm [36] which does not allow for the lessening of back tension due to capillary oxygen unsaturation. The correction in Dco, from 3.7 to 3.9 was thus an overestimate. It is insignificant.

The smallness of the error introduced by the back tension of CO in these cases emphasises the reduction of Dco. The mean normal value in this laboratory in a somewhat younger age group was 22 ml./min./mm. Hg [19]. This and most of the series on normal subjects published are uncorrected for back tension and the normal Dco values should be higher; the figures in these patients are much less in error from this effect.

The low values of Dco are even more significant as it is the diffusing capacity of the lung as a whole (D_L) which is here estimated. In normal subjects, it appears that D_L is less than half the diffusing capacity of the alveolocapillary membrane (D_M) ; there is considerable intracapillary resistance to diffusion of CO caused by its finite rate of combination with hemoglobin [27,34,37]. At the lowered values of pulmonary capillary oxygen tension in this condition, the intracapillary resistance to CO uptake is reduced and D_L approaches D_M .

Thus D_M is greatly reduced when compared with the normal value of about 50 ml. CO/min./ mm. Hg [27]. It is possible that one of our patients (W. S.) had a value of DMCO only onetenth of this, as the value found for his D_LCO was as low as 3.6 ml./min./mm. Hg. However, the fall in D_L may not all be caused by the fall in D_M as a lowering of pulmonary capillary blood volume also causes a fall in D_L [27]. McNeill, Rankin and Forster [38], using the breath-holding technic [17] for DLCO, found that the pulmonary capillary blood volume was reduced in "fibrosis," this reduction playing a minor part in causing the fall in D_L . The lowering of D_M in the Hamman-Rich syndrome is attributed mainly to the great thickening of alveolar walls which occurs, and partly to a decrease in diffusing surface caused by obliteration of capillaries.

A comparison with breath-holding Dco was not made in any of these cases. In the cases previously studied by this method [17] Dco was severely reduced. In miscellaneous cases of alveolocapillary block Marks et al. [13] found the breath-holding Dco to be significantly higher, and suggested possible reasons for this. A discussion of the findings of other investigators is beyond the scope of this paper but it should be pointed out that inequalities of A-c membrane thickness and of blood flow should cause inequalities of the ratio

diffusing capacity alveolar volume

on whose uniformity the validity of the breath-holding method depends [31], just as they cause inequalities of the ratio diffusing capacity/alveolar ventilation. Nor is it certain that a representative sample will be delivered. For these reasons the breath-holding method is liable to error in the Hamman-Rich syndrome.

D_LCO rose on exercise in all the cases studied. This rise is not necessarily due to the opening up of fresh capillaries to give an increased diffusing surface; it may be largely due to the decreased oxygen tension on exercise of pulmonary capillary blood. This will result in the speeding up of the reaction between CO and hemoglobin and permit D_LCO to rise without any increase in diffusing surface. Although the rise in diffusing capacity is parallel to the normal regression line, the rise could be regarded as more abrupt than in normal subjects. The resting values were lower than in normal subjects and so the in-

Table v
Oxygen tensions, breathing reserve and dyspnea levels on exercise

Patient	O ₂ Consumption (ml./min.)	Dco (ml./min./mm. Hg)	DO ₂ (ml./min./mm. Hg)	Mean Alveolo- capillary O ₂ Gradient (mm. Hg)	Alveolar O ₂ Ten- sion (mm. Hg)	Mean Capil- lary O ₂ Ten- sion ,(mm. Hg)	Arterial O ₂ Tension (mm. Hg)	Breathing Reserve (%)	Dyspnea After Exercise
W. J.	593	8.0	9.9	60	107	47	42	62	Severe
D. C.	827	10.9	13.4	62	117	55	59	55	Severe
	603	9.9	12.2	49	113	64	68	65	Mild to moderate
	784	11.2	13.8	57	114	57	50	51	Maximal
S. K.	552	7.9	9.7	57	105	48	64	37	Moderate to sever
R.F.	506	9.6	11.8	43	105	62	65	72	Mild to moderate
W.S.	443	5.7	7.0	63	118	55	45	68	Mild
	598	8.1	10.0	60	127	67	39	27	Maximal
	505	6.6	8.1	62	113	51	36	59	Mild to moderate
	575 -	7.1	8.7	66	116	50	39	46	Very severe

crease, considered as a fraction of the resting value, was greater in these patients. For instance, the Dco in one case (W. S.) reached a value double the resting figure at an oxygen consumption of less than 600 ml./minute. It is not possible to know to what extent the various possible mechanisms operated in producing this increase. The rapid rise in Dco, as measured, may have been due to correction of non-uniformity on exercise.

Luchsinger et al. [39] emphasised the importance of capillary destruction and of fixed arteriolar resistance in the production of arterial oxygen unsaturation on effort in many clinical conditions. They suggest that this, with its consequent reduction in alveolocapillary contact time, is important, rather than what they term "membrane factor." This suggestion is misleading. It must be recognised that the diffusing capacity can be decreased by reduction of effective A-c membrane surface area just as surely as it can be reduced by increase in thickness of the membrane. Both of these must be regarded as contributing to a membrane factor although with the tests usually performed they cannot be distinguished.

To assess the adverse effect of the lowering of the diffusing capacity, the mean oxygen tension gradient between the alveoli and the pulmonary capillary blood was calculated. In order to do this the diffusing capacity for carbon monoxide has to be converted to diffusing capacity for oxygen. If the diffusing capacities are related by the formula

$$D_{0_2} \times 1.23 = D_{CO}$$

derived from a knowledge of the molecular weight and the water solubility of the two gases, the conversion presents no difficulty. Since the

demonstration that the diffusing capacity of the lung as a whole is largely dependent on the rate of reaction of the gas in question with hemoglobin [34] this relationship cannot be expected to hold fully. As the capillary tension of oxygen falls, the intracapillary resistance to the uptake of gas also falls, the diffusing capacity of the lung approaches that of the membrane, and the aforementioned conversion formula becomes more accurate. On exercise, when the pulmonary capillary tension of oxygen is lower, the gradients and tensions have been calculated as follows: (1) D_{CO} multiplied by 1.23 to give Dos; (2) mean alveolocapillary oxygen gradient by dividing oxygen consumption by Do, (follows from the definition of diffusing capacity); (3) alveolar oxygen tension from the alveolar air equation or the charts of Rahn and Fenn [40]; (4) mean capillary oxygen tension by subtracting "2" from "3"; (5) arterial oxygen tension from the hemoglobin dissociation curve of Riley [41] or the chart of Severinghaus [42]. The figures obtained are shown in Table v. None of the patients showed the impossibly high alveolocapillary gradients found by Bates [16]; in fact all the gradients came within a comparatively small range with 66 mm. Hg as the upper limit.

The reasonable values of all the A-c gradients in this series, even in cases exercising near their limit, suggests strongly that the Filley method does not seriously underestimate Dco in this condition, despite the presence of some non-uniformity in the lungs. As shown by Bates, the use of an assumed dead space does lead to an impossibly high gradient, and for comparison the present results were recalculated with a dead space assumption.

The values taken were according to the standards of Radford [43] which Bates [16] used for

Table VI

THE VALUES WHICH WOULD BE FOUND ON EXERCISE FOR DIFFUSING CAPACITY AND ALVEOLO-CAPILLARY OXYGEN GRADIENT USING AN ASSUMED DEAD SPACE*

Patient	Weight (lb.) = Assumed Dead Space (ml.)	Dead Space as Found (ml.)	Tidal Volume (ml.)	O ₂ Consumption (ml./min.)	Dco (by assumed dead space) (ml./min./mm. Hg)	A-c Tension Gradient for O_2 (by assumed V_D) (mm. Hg)
W. J.	133	502	866	593	7.5	79
D. C.	126	827	1906	849	9.3	73
	121	603	1553	797	7.8	63
		784	1576	841	9.1	70
S. K.	88	174	450	552	7.3	61
R. F.	121	312	700	509	7.5	55
		307	708	504	7.1	58
W.S.	152	559	1095	443	4.7	76
		916	1616	598	6.9	70
	153	782	1309	505	5.3	78
		1028	1636	575	5.7	82

^{*} See text.

estimation of Dco in this condition. Dead space (ml. BTPS) is taken as equal to body weight in pounds and 70 ml. is added for the mouthpiece. Table v1 shows the results so obtained. In every case the dead space was too low, leading to underestimation of the diffusing capacity and overestimation of the A-c gradient. (Compare with Table v.) The error introduced into the Dco and gradient is higher in those subjects with a large physiological dead space, although not as large as would be caused in normal subjects. The assumption of a dead space almost certainly accounts for much of the excessive gradient found by Bates.

The Filley diffusing capacity thus appears a valid estimation in cases of the Hamman-Rich syndrome; its lowering gives a fair correlation with severity of the disease, although much of the disability is due to increased dead space and much of the arterial oxygen unsaturation to venous admixture. It would not be correct to expect a complete correlation between the lowering of Dco and disability; diffusing capacity gives an estimate of the ease with which gases pass from the alveoli to the blood but there is no indication that diffusion difficulty is correlated more than in a general way with the increase in dead space or venous admixture which are such a large cause of disability.

Fractional CO Uptake (CO_F). This measurement clearly distinguished the patients from the normal subjects previously studied here [19].

As it depends on diffusion, dead space and level of ventilation, it is not useful in investigation of the physiological abnormality. Its dependence on dead space as well as on diffusion gives it a good correlation with disability.

Venous Admixture. The venous admixture was not measured in any of the cases but in many cases a good estimate of its magnitude can be made from the information available. With a knowledge of the alveolar oxygen tension $(P_{A_{O_2}})$, the mean alveolocapillary oxygen gradient, and the arterial oxygen tension (P and), the venous admixture can be calculated from the charts of Riley et al. [44] provided the value for the difference of oxygen saturation between endcapillary and mixed venous blood (SceO2 - $S\bar{v}_{02}$) is known. As there was no way of estimating this difference, the value for venous admixture was calculated using all the values of the difference for which charts were available, that is, for a range of $Sc^{e}_{O_2} - S\bar{v}_{O_2} = 15$ to $Sc^{e}_{O_2} S\bar{v}_{O_2} = 70$. The calculation was made only for studies on exercise for two reasons. First, as explained above, the conversion of Dco to Do. is more reliable at the lower capillary oxygen tensions found on exercise; and secondly, higher arterial oxygen saturations found at rest make the calculations of arterial oxygen tension very liable to error. The results obtained were as follows. One patient (W. J.) exercising at an oxygen consumption (V_{O2}) of 593 ml./minute

had a value for venous admixture (Qva/Qt) of at least 30 per cent. If the venous admixture is recalculated for an arterial oxygen saturation in error by 2 per cent, Qva/Qt is at least 26 per cent. In the case of D. C. the only occasion for which venous admixture could be calculated with any reliability was for $V_{O_2} = 784 \text{ ml./min-}$ ute. Qva/Qt was at least 22 per cent. In case S. K., exercising at $V_{O_2} = 552$ ml./minute, Ova/Ot was very low and recalculation to allow for a 2 per cent error in arterial oxygen saturation gave a value for Qva/Qt of, at the most, 15 per cent. In case R. F. no reliable calculations could be made but in the case of W. S. high values were found. On exercise, where Vo2 = 443, Qva/Qt was at least 28 per cent; where $\dot{V}_{O_2} = 598$, $\dot{Q}va/\dot{Q}t$ was at least 38 per cent; where $V_{02} = 505$, Qva/Qt was at least 47 per cent; and where $\dot{V}_{O_2} = 575$, $\dot{Q}va/\dot{Q}t$ was at least 37 per cent. The calculations show that in no case does a defect in diffusion alone account for a serious degree of arterial desaturation, and that there is a high venous admixture in the more severe cases. In case S. K. there was a much lower figure for venous admixture ratio. She also had a lower dead space/tidal volume ratio than the other patients so physiologically her nonuniformity was less on both counts. This correlates with the histologic findings, this patient being the only one known not to have fine diffuse emphysema at the time of testing. The finding of a greatly raised dead space and venous admixture in cases with a marked diffusion defect is in accord with the previous observations of Austrian et al. [11] and Donald et al [12].

Ventilation and the Causation of Dyspnea. Much of the increase in ventilation in these cases is due to the increase in physiological dead space, alveolar ventilation usually being near to normal. The excessive dead space on exercise involves an increase of the ventilation so that the dyspnea is reached at lower levels of alveolar ventilation. The severity of the dyspnea is fairly well related to reduction in the breathing reserve (Table v) although not as well as in the series of mixed fibroses of Baldwin et al. [10]. However, the effect of oxygen breathing on exercise in cases D. C. and W. S. indicates that the anoxemia necessitates increased ventilation and so the diffusion impairment and excessive venous admixture both play a part in the causation of dyspnea at relatively low exercise levels.

Alveolar Ventilation and the Stimulus to Breathing. Only two patients had alveolar hyperventilation at rest. It was shown to be due to nervousness in one (D. C.) and to be abolished by oxygen breathing in the other (W. S.). The alveolar hyperventilation in W. S. is therefore attributed to a hypoxic stimulus, possibly via the chemoreceptors, rather than to sensitization of the reflex receptors in the lung by the fibrotic process as has been suggested by Baldwin et al. [10] and by Marks et al. [13]. Similarly the fall in ventilation in D. C. and W. S. when given oxygen during exercise (Table IV) is attributed to the removal of anoxic drive. A great deal of this fall, of course, was due to decrease in dead space ventilation concomitant with the fall in alveolar ventilation. The failure of alveolar ventilation to rise in W. J. as oxygen saturation fell may have been due to the disease being too advanced to permit a rise.

The Limitation of Effort. In all patients who exercised more than minimally, the limit to effort was dyspnea at a comparatively light level of work. The causation of dyspnea on light exercise has been discussed but the effect of lowered diffusing capacity has not been considered. This is best approached in the light of the work of Shepard [45] who showed that diffusion impairment limits exercise rapidly when a critical level of oxygen consumption is reached. On applying his graphs to the results of the patients studied, W. S. is found to be at the critical level, and W. J. close to it. Although both patients had a lot of venous admixture in each case the diffusion defect had set a low limit to exercise.

exercise.

The studies presented herein have shown that in the Hamman-Rich syndrome the diffusion defect is severe but is usually accompanied by an increase in physiologic dead space and venous admixture. Any study of this condition which estimates the diffusing capacity but neither of these manifestations of non-uniformity will give a false impression of the disordered function present. Although the diffusion defect does lead to serious disability, the effect on the patient of the increased dead space and venous admixture is also great, and must be recognised as a vital part of the disease.

SUMMARY

Pulmonary function was studied in five patients with the Hamman-Rich syndrome.

The spirometric tests showed a reduction in

lung volume with no subdivision particularly affected; the maximum breathing capacity varied, being normal or reduced.

The increased ventilation was due largely to an increase in the physiological dead space, the alveolar ventilation at rest usually being normal. In the one patient with a true resting alveolar hyperventilation, this was relieved by oxygen. Exercise ventilation also decreased with the administration of oxygen. No support was found for the hypothesis of increased sensitivity of a pulmonary reflex.

The validity of the Filley method for determination of Dco in the Hamman-Rich syndrome was considered. It appears to be the most suitable method for use in this condition, particularly if exercise studies are to be made.

Dco (Filley method) was greatly lowered in all patients at rest and on exercise. The lowering accounts for some of the arterial oxygen unsaturation in severe cases and, in general, correlates well with the severity. The rise of the diffusing capacity on exercise was parallel to the normal regression line.

Resting arterial oxygen saturation was normal or reduced, depending on the severity of the case. In all cases the saturation fell on exercise. There was no consistent pattern of blood CO₂. In no case was the diffusion defect great enough to account for a marked reduction of arterial oxygen saturation; when this was present there was an increase in venous admixture.

Impaired diffusion, increased physiological dead space, and increased venous admixture all play a part in the causation of the disability in the Hamman-Rich syndrome.

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REFERENCES

- Намман, L. and Rich, A. R. Fulminating diffuse interstitial fibrosis of the lungs. Tr. Am. Clin. & Climatol. A., 51: 154, 1935.
- HAMMAN, L. and RICH, A. R. Acute diffuse interstitial fibrosis of the lungs. Bull. Johns Hopkins Hosp., 74: 177, 1944.

- Rubin, E. H. and Lubliner, R. The Hamman-Rich syndrome: review of the literature and analysis of 15 cases. *Medicine*, 36: 397, 1957.
- FORBES, I. J. Diffuse interstitial pulmonary fibrosis and honeycomb lung. Australasian Ann. Med., 7: 205, 1958.
- READ, J. R. The pathogenesis of the Hamman-Rich syndrome: a review from the standpoint of possible allergic etiology. Am. Rev. Tuberc., 78: 353, 1958.
- SILVERMAN, J. J. and TALBOT, T. J. Diffuse interstitial pulmonary fibrosis camouflaged by hypermetabolism and cardiac failure: antemortem diagnosis with biopsy and catheterization studies. Ann. Int. Med., 38: 1326, 1953.
- MERRILL, J. M., CALLAWAY, J. J. and MENEELY, G. R. Diffuse interstitial fibrosis of the lungs (Hamman-Rich syndrome): a case report with pulmonary function studies. South. M. J., 49: 997, 1956.
- FLEISHMAN, S. J., BOSMAN, A. R. and FULLER, D. N. Diffuse interstitial fibrosis of the lungs: successful treatment of a case, with adrenal steroids. Am. J. Med., 24: 823, 1958.
- 9. READ, J. R. and HOLLAND, R. A. B. Treatment of the Hamman-Rich syndrome with cortisone. *Thorax*, 14: 71, 1959.
- Baldwin, E. De F., Cournand, A. and Richards, D. W., Jr. Pulmonary insufficiency. II. A study of 39 cases of pulmonary fibrosis. *Medicine*, 28: 1, 1949.
- AUSTRIAN, R., McCLEMENT, J. H., RENZETTI, A. D., JR., DONALD, K. W., RILEY, R. L. and COURNAND, A. Clinical and physiological features of some types of pulmonary disease with impairment of alveolar-capillary diffusion: the syndrome of alveolar-capillary block. Am. J. Med., 11: 667, 1951.
- 12. Donald, K. W., Renzetti, A., Riley, R. L. and Cournand, A. Analysis of factors affecting concentrations of oxygen and carbon dioxide in gas and blood of lungs: results. J. Appl. Physiol., 4: 497, 1952.
- MARKS, A., CUGELL, D. W., CADIGAN, J. B. and GAENSLER, E. A. Clinical determination of the diffusion capacity of the lungs: comparison of methods in normal subjects and patients with "alveolar-capillary block" syndrome. Am. J. Med., 22: 51, 1957.
- LÉPINE, C., LABERGE, M. J., LAPALME, J., SOUCY, R., BORDUAS, J. L. and GRÉGOIRE, F. La fibrose pulmonaire, *Union méd.*, *Canada*, 86: 144, 1957.
- CUGELL, D. W., MARKS, A., ELLICOTT, M. F., BADGER, T. L. and GAENSLER, E. A. Carbon monoxide diffusing capacity during steady exercise: comparison of physiologic and histologic findings in patients with pulmonary fibroses and granulomatoses. Am. Rev. Tuberc., 74: 317, 1956.
- BATES, D. V. The measurement of pulmonary diffusing capacity in the presence of lung disease. J. Clin. Invest., 37: 591, 1958.
- OGILVIE, C. M., FORSTER, R. E., BLAKEMORE, W. S. and MORTON, J. W. A standardised breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide.
 J. Clin. Invest., 36: 1, 1957.
- 18. HOLLAND, R. A. B. Physiologic dead space in the

Hamman-Rich syndrome: physiological and clinical implications. Am. J. Med., 28: 61, 1960.

 HOLLAND, R. A. B. and BLACKET, R. B. The carbon monoxide diffusing capacity of the lung in normal subjects. Australasian Ann. Med., 7: 192, 1958.

 McMichael, J. A rapid method of determining lung capacity. Clin. Sc., 4: 167, 1939.

 NEEDHAM, C. D., ROGAN, M. C. and McDonald, I. Normal standards for lung volumes, intrapulmonary gas mixing, and maximum breathing capacity. *Thorax*, 9: 313, 1954.

 FILLEY, G. W., MACINTOSH, D. J. and WRIGHT, G. W. Carbon monoxide uptake and pulmonary diffusing capacity in normal subjects at rest and during exercise. J. Clin. Invest., 33: 530, 1954.

 ROSENTHAL, T. B. The effect of temperature on the pH of blood and plasma in vitro. J. Biol. Chem., 173: 25, 1948.

24. Van Slyke, D. D. and Neill, J. M. The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. J. Biol. Chem., 61: 523, 1924.

 SINGER, R. B. and HASTINGS, A. B. Improved clinical method for estimation of disturbances of acid-base balance of human blood. *Medicine*, 27: 223, 1948.

- Pappenheimer, J. R., Comroe, J. H., Cournand, A., Ferguson, J. K. W., Filley, G. F., Fowler, W. S., Gray, J. S., Helmholz, H. F., Otis, A. B., Rahn, H. and Riley, R. L. Standardization of definitions and symbols in respiratory physiology. *Fed. Proc.*, 9: 602, 1950.
- 27. ROUGHTON, F. J. W. and FORSTER, R. E. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with especial reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J. Appl. Physiol., 11: 290, 1957.
- READ, J. R. Intrapulmonary gas mixing studies by the closed circuit helium technique. II. Normal and abnormal states in man. Australasian Ann. Med., 7: 187, 1958.
- Gilson, J. C. and Hugh-Jones, P. Lung function in coalworkers' pneumoconiosis. London, 1955. Her Majesty's Stationery Office.

 BATES, D. V., BOUCOTT, N. G. and DORMER, A. E. The pulmonary diffusing capacity in normal subjects. J. Physiol., 129: 237, 1955.

 FORSTER, R. E., FOWLER, W. S. and BATES, D. V. Considerations on the uptake of carbon monoxide by the lungs. J. Clin. Invest., 33: 1128, 1954. HOFF, H. R. The Hamman-Rich syndrome: review of the literature and report of a case treated with prednisone. New England J. Med., 259: 81, 1958.

 FORSTER, R. E. Exchange of gases between alveolar air and pulmonary capillary blood: pulmonary diffusing capacity. *Physiol. Rev.*, 37: 391, 1957.

- FORSTER, R. E., ROUGHTON, F. J. W., CANDER, L., BRISCOE, W. and KREUZER, F. Apparent pulmonary diffusing capacity for CO at varying alveolar O₂ tensions. J. Appl. Physiol., 11: 277, 1957.
- ROUGHTON, F. J. W. and ROOT, W. S. The estimation of small amounts of carbon monoxide in blood. J. Biol. Chem., 160: 123, 1945.
- LINDERHOLM, H. On the significance of CO tension in pulmonary capillary blood for determination of pulmonary diffusion capacity with the steady state CO method. Acta med. scandinav., 156: 413, 1957.
- LEWIS, B. M., LIN, T-H., NOE, F. E. and KOMISARUK, R. The measurement of pulmonary capillary blood volume and pulmonary membrane diffusing capacity in normal subjects; the effects of exercise and position. J. Clin. Invest., 37: 1061, 1958.

 McNeill, R. S., Rankin, J. and Forster, R. E. The diffusing capacity of the pulmonary membrane and the pulmonary capillary blood volume in cardiopulmonary disease. Clin. Sc., 17: 465, 1958.

- Luchsinger, P. C., Moser, M., Bühlmann, A. and Rossier, P. H. The interrelationship between cor pulmonale, capillary bed restriction and diffusion insufficiency for oxygen in the lung. *Am. Heart J.*, 54: 106, 1957.
- RAHN, H. and FENN, W. O. A graphical analysis of the respiratory gas exchange: the O₂—CO₂ diagram. Washington, D. C., 1955. The American Physiological Society.
- RILEY, R. L. and COURNAND, A. "Ideal" alveolar air and the analysis of ventilation-perfusion relationships in the lungs. J. Appl. Physiol., 1: 825, 1949.
- SEVERINGHAUS, J. W. Oxyhemoglobin dissociation curve correction for temperature and pH variation in human blood. J. Appl. Physiol., 12: 485, 1958.
- RADFORD, E. P. Ventilation standards for use in artificial respiration. J. Appl. Physiol., 7: 451, 1955.
- RILEY, R. L., COURNAND, A. and DONALD, K. W. Analysis of factors affecting partial pressures of oxygen and carbon dioxide in gas and blood of lungs: methods. J. Appl. Physiol., 4: 102, 1951.

 SHEPARD, R. H. The effect of pulmonary diffusing capacity on exercise tolerance. J. Appl. Physiol., 12: 487, 1958.

Antibiotics and Terminal Pneumonia*

A Postmortem Microbiological Study

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For well over half a century bacteriological studies of the human lung at autopsy have been reported in the medical literature with a view to elucidating the etiology and pathogenesis of terminal bronchopneumonia. In 1947 Smillie and Duerschner [7,2] described the bacteriological findings in over 200 carefully studied cases. In brief, their conclusions were as follows:

Cultures taken at autopsy from the trachea at its bifurcation always show growth, and the flora obtained are a qualitative replica of the pharyngeal flora, even when death is virtually instantaneous, as in electrocution. These investigators believed, however, that this phenomenon was agonal, because ten of fourteen cultures obtained through a bronchoscope in living persons were sterile. Thus the recovery of bacteria from the tracheobronchial tree at autopsy, even though potential pathogens were found, appeared to be without significance although they were considered "potential invaders." On the other hand, their studies indicated that the recovery of organisms from lung tissue was of significance and constituted invasion. These organisms were not ordinarily the inconsequential inhabitants of the mouth and throat; they were the potential pathogens and more often than not associated with histologic evidence of bronchopneumonia. Smillie and Duerschner concluded that "pneumococci, influenza bacilli, and beta type hemolytic streptococci were of greatest potential danger in the production of terminal bronchopneumonia." In regard to Staphylococcus aureus they pointed out that it was found more frequently in persons with terminal pneumonia than any other organism, but it was also found frequently in persons without pneumonia. In conclusion they stated, "We have evidence that in certain individuals Staph. aureus did produce a terminal bronchopneumonia, but this organism was not as important as the other three organisms."

Smillie's cases were studied at a time when sulfonamides were in use; penicillin, however, was just beginning to be available on an adequate scale. The decade which has elapsed since the publication of these monumental papers has been characterized by extraordinary developments in the field of antibiotic medicine. Powerful new agents have been introduced, and their widespread use has apparently altered the old established patterns of microbial infection. It was this fact which led us to re-explore a field which had been so carefully investigated in the past—this, together with a desire to learn something more about the influence of antibiotics which are now so commonly prescribed in large amounts up to the moment of death. It is obvious that any conclusions as to the efficacy of drug therapy based exclusively on autopsy material must be drawn with extreme caution. Nevertheless it was thought that some new light might be shed on the vexed problem of "prophylactic" antibiotic usage. Lastly, it was hoped that studies of higher bacteria and attempts at virus isolation from lung tissue might yield information of interest.

MATERIALS AND METHODS

This study is based on material obtained from 201 autopsies performed at the Columbia-Presbyterian Medical Center in New York City. The cases were selected only in that the autopsies included were performed during the hours of the normal working day between Monday and Saturday. The period covered is from October 9, 1956 to January 21, 1958, and all services were represented except pediatrics.

The clinical records of all patients were carefully abstracted. Particular attention was paid to the antibiotic therapy, which was recorded in precise detail.

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Table 1
PNEUMONIA AT AUTOPSY: NOW AND THIRTY YEARS AGO

	Present Series (%)	1928–1929 Cases (%)
Pneumonia deaths	8.5 21.5 25.0	10.5 20.0 31.0
Pneumonia non-contributory No pneumonia	45.0	38.5

Cultures of the trachea were obtained from swabs contained in a sterile glass tube inserted through an incision. The swabs were streaked on rabbits' blood agar and also rubbed up in a tube of blood broth. After incubation for four to six hours, an injection of 0.5 ml. of the broth culture was given intraperitoneally to a mouse. Lung cultures were obtained from the lower lobes or any area grossly suggestive of pneumonia. The pleural surface was thoroughly seared and a sizable piece of lung removed with sterile instruments. The specimen was then bisected and half was dipped in boiling water for not more than five seconds. Thereafter both pieces were treated in identical fashion so that the cultural procedures were carried out in duplicate. To obtain a rough quantitative indication of the number of organisms present, pieces were cut from the deep part of each specimen with sterile instruments, and direct impressions of a cut surface approximately 1 by 1 cm. in size were made on rabbits' blood agar plates and also on Sabouraud's agar plates. The latter were kept at room temperature for a month. In addition, pieces of approximately 1 gm. were put in tubes of blood broth. From four to six hours later an injection of 0.5 ml. of one of these was given intraperitoneally to a mouse. The blood broth cultures were incubated at 39.5°c. for two days, and kept at room temperature for one week before being read as negative.

Three to 5 ml. of the heart's blood obtained after searing the right ventricle were put in a flask containing 100 ml. of broth, and 1 ml. was placed in a 5 ml. broth tube. These cultures were incubated for a week (and longer if bacterial endocarditis was suspected). The heart's blood was also cultured on Sabouraud's agar.

Gram-positive cocci were identified in the usual way. All staphylococci were tested for coagulase production on 20 per cent human plasma. Suspected enterococci were grown in 6.5 per cent NaCl broth. Gram-negative rods were classified according to their reactions on Krumwiede's triple sugar, Simmons' citrate agar and on urea broth. They were also tested for motility and on TS1 agar and gelatin when necessary.

Antibiotic sensitivities were determined by the use of Difco Bactodiscs, penicillin 10 units, streptomycin 100 μ g., Achromycin® 30 μ g., Chloromycetin® 30 μ g. and erythromycin 15 μ g. Gram-positive cocci were tested against all five, gram-negative bacilli against streptomycin, Achromycin and Chloromycetin. Staphylococci were seeded from six-hour cultures, the other organisms from twenty-four-hour cultures.

RESULTS

It was initially thought of interest to divide our series into four broad categories, i.e., patients dying of pneumonia, patients dying with pneumonia as a contributory cause, those in whom pneumonia was discovered as an incidental autopsy finding, and those without pneumonia. We then compared these figures with a series of 200 consecutive autopsies performed at Presbyterian Hospital in 1928-1929. At that time, the only available antibacterial agent was type I antipneumococcal horse serum. However, as nearly as one can tell by a study of the autopsy protocols and the excellent photomicrographs appended thereto, the criteria for the histologic diagnosis of pneumonia have not significantly altered in the past thirty years. The results of this comparison are given in Table 1.

One is immediately impressed by the similarity of these figures, particularly when it is remarked that 68 per cent of the present series of patients were under antibiotic treatment. However, in considering the "pneumonia deaths" an important correction must be made. From the lungs of eight patients dving of pneumonia in October 1957, Asian influenza virus with or without Staph. aureus was recovered. This was a very special circumstance, as it occurred at the height of a pandemic. If one corrects the figure for deaths from pneumonia in the present series by subtracting the cases of influenzal pneumonia, the percentage falls to 4.5 per cent-a trifle less than half that found in 1928–1929. Most of the cases in the earlier group appeared to be "old-fashioned" lobar pneumonia. It is not surprising that the likelihood of dying of lobar pneumonia today has been substantially reduced. However, that there should be so little change in the figures for "pneumonia contributory" and "pneumonia noncontributory" in this era of antibiotics gives one food for thought.

In order to inquire further into the relationship of antibiotics and pneumonia, we have divided our series into those patients treated with antibiotics up to death or shortly before it,

and those who received no antibiotics for a week prior to death. The incidence of pneumonia at autopsy in the two groups is given in Table II.

As can be seen, slightly more than two-thirds of patients coming to autopsy nowadays do so while under antibiotic therapy, and the percentage of these having histologic evidence of pneumonia is higher than in the untreated patients. This surprising statistic cannot, of course, be construed as evidence that antibiotics increase the likelihood of pneumonia. After all, the great majority of these patients were suffering from some lethal chronic disease. Many had clinical evidence of terminal pneumonia, were treated as a forlorn hope, and died of the underlying condition nonetheless. It does indicate, however, that antibiotics, even in massive dosage and wide combination, are no insurance against infection of the lungs.

Before proceeding to a more detailed consideration of our bacteriological material, a word or two might be said about the significance of finding bacteria in the lungs at autopsy. It may be recalled that Smillie and Duerschner concluded that they were of significance. Our data would tend to support this. Table 11 indicates that sixty-three patients were not treated with antibiotics. Of these, thirty-one had pneumonia at the time of death, and all but one of the lung cultures yielded abundant growth of bacteria. On the other hand, 33 per cent of the lungs which showed no pneumonia were bacteriologically sterile. Interestingly enough, the percentage of sterile lungs in treated patients showing no pneumonia was precisely the same as in the untreated patients-33 per cent.

Up to this point these rough quantitative comparisons would seem to indicate that antibiotic therapy had a negligible influence on the finding of pneumonia at autopsy. It is not until one considers the specific organisms involved that a striking change in pattern, attributable to antibiotics, becomes clearly apparent. Perhaps the best way to make a meaningful presentation of our voluminous data is to consider the subject according to the organisms found.

Pneumococcus, Hemophilus Influenzae, Hemolytic Streptococcus. In the past, the pneumococcus, as its name implies, has been considered the chief incitant of acute inflammation of the lungs in man. It is the sole etiologic agent in typical lobar pneumonia. In terminal bronchopneu-

TABLE II
PNEUMONIA AT AUTOPSY WITH AND WITHOUT ANTIBIOTICS

	Cases (no.)	Pnemo	eu- nia	Pnemo	eu-
		No.	%	No.	%
With antibiotics	136 65	79 31	58 48	57 33	42 52

monia it will be recalled that Smillie and Duerschner considered it the principal pathogen. They found it in forty of 109 cases, in pure culture in twenty-five. With regard to lobar pneumonia, they found pneumococcus in all eight cases. Our figures are strikingly different.

Seventeen of our patients were considered to have died "of" pneumonia. In only one of these were pneumococci recovered. This patient (who had metastatic carcinoma and received, mercifully, no antibiotics during the last ten days of life) showed lobar pneumonia at autopsy with type 8 pneumococcus recovered from the lungs and heart's blood. However, the pneumococcus played a larger role in patients dying "of" pneumonia than this single figure would indicate. In five other cases there was clinical evidence of pneumococcal pneumonia to begin with (four established, one presumptive); all these patients were treated with antibiotics, and no pneumococci could be recovered at autopsy. Other organisms, however, were found-pyocvaneus, Klebsiella-aerogenes, Escherichia coli, Clostridium welchii, enterococci. Thus it seems probable that the pneumococcus can initiate pneumonia and be eliminated by therapy, leaving other bacteria to carry on their work. How often this occurs cannot be accurately estimated from our data but it may be presumed to be fairly uncommon.

Much the same situation exists in regard to those cases in which pneumonia was deemed a "contributory" cause of death. In forty-four of these, pneumococci were recovered only thrice. The types were 13, 14 and 19; all three patients had malignant disease and none had received antibiotic therapy. Lastly, among forty-nine cases in which pneumonia was considered an incidental postmortem finding, pneumococci were also recovered thrice, again in untreated patients. Thus pneumococci were recovered

altogether seven times from the total series of 110 cases of pneumonia. From these findings it can reasonably be concluded that "old fashioned" lobar pneumonia as a cause of death in the antibiotic era has approached the vanishing point in a large general hospital, and that the pneumococcus, formerly the principal threat to pulmonary tissue in persons suffering from a variety of chronic diseases, is of far less importance than formerly. This can be attributed to the fact that the pneumococcus is uniformly sensitive to virtually all the antibiotics, and resistant forms have not yet appeared in man. That its place has been taken by other disease agents will become clear later on.

Following the pneumococcus, the next two organisms cited by Smillie and Duerschner as threatening to produce terminal pneumonia by invading lung tissue from the tracheobronchial tree were H. influenzae and beta hemolytic streptococci. In our series, these can be disposed of briefly. H. influenzae was recovered only eleven times (despite technic especially aimed at its isolation). In six of these instances there was no associated pneumonia. In other words, this organism was more likely to be recovered from the lung of patients without pneumonia. It was never found in pure culture, and growth was often scanty. Only two patients from whom it was recovered had received antibiotics.

Group A hemolytic streptococcus was never encountered.

Thus one can generalize about the traditional pulmonary mischiefmakers with a single word: *Exeunt*. One must qualify this statement in regard to the pneumococcus; in view of the fact that it was recovered from the trachea in seven instances (in addition to the seven recoveries from lung tissue) one may conclude that, so to speak, it remains "in the wings," and in the absence of antibiotics would return to the stage.

If, as has been pointed out, the over-all figures for terminal pneumonia have not materially changed in thirty years, different organisms must have appeared to take the place of the traditional ones. We shall now try to suggest, in the light of our data, what these may be, taking up each organism in turn. As these are considered individually, answers to certain questions will be sought: Does the organism indeed cause bronchopneumonia? How great a threat does it constitute? Has its role changed in importance in recent years? If so, how far can this be attributed to anti-

biotics? Have antibiotics, in fact, done good or harm? An attempt will be made to answer these questions by a consideration of the bacteriologic findings in the lungs, as nearly as possible in quantitative terms for each organism, comparing those lungs which showed pneumonia with those which did not. In addition the significance of organisms recovered from the heart's blood will be discussed.

In the ensuing paragraphs the term "antibiotics" is freely used without specific indication as to which ones are meant. The reason for this is that among 136 patients treated with antibiotics, penicillin was employed as the sole agent in only nine. In this series, therefore, antibiotic therapy was almost invariably of the broad-spectrum type.

Micrococcus Pyogenes Var. Aureus (Coagulase Positive). The first organism to be considered will be Staph. aureus. This selection is made not because of the current interest in staphylococcal infections but because, as will become evident, it proved to be more frequently encountered than any other bacterium. A synopsis of some of our data in regard to the staphylococcus is given in Table III (section A).

A consideration of the percentages in Table III (section A) indicates that staphylococci are found nearly twice as frequently in patients whose lungs show pneumonia at autopsy as in those without pneumonia. This one comparison would suggest some etiologic significance of the staphylococcus in bronchopneumonia. On the other hand, if the organism is recovered at all, the likelihood of its being present in large numbers is not much greater in patients with pneumonia than in those without. The reasons for this finding are not entirely clear. It cannot be ascribed to postmortem growth, as it was unrelated to the time elapsing between death and autopsy. Presumably it reflects the fact that the organism is not a specialized pulmonary pathogen in the sense that the pneumococcus is.

The figures just cited are not terribly incriminating evidence against the staphylococcus. Still it will be noted that staphylococci were only once recovered in pure culture in the absence of pneumonia, as opposed to ten times in its presence. Moreover, when one reviews our cases in which pneumonia was the sole cause of death, or an important contributory cause, and in which the disease was believed to be due to a single organism (i.e., when it was not believed to be a mixed infection), the staphylococcus was

implicated in seventeen. This is a far higher figure than was achieved by any other organism, as can be seen in Table v.

Lastly, one comes to the subject of heart's blood cultures. Such cultures were made in 141 cases in our series. Fifty were positive, and of these positive cultures seventeen showed staphylococci, a larger score than for any other bacterium. The fact that fifteen of these seventeen patients with staphylococci in the blood had pneumonia is additional evidence that the organism is of real significance in bronchopneumonia at the present time. There is no striking suggestion, however, that we are dealing with a staphylococcal epidemic which has gotten beyond control. The incidence of the finding of staphylococci at autopsy has undoubtedly increased in recent years, but the increase is probably not very great. Smillie found staphylococcus in 38 per cent of his pneumonia cases. In our series it was recovered in 50 per cent. This moderate increase is presumably a result of the widespread use of antibiotics with two mechanisms operatingfirst the virtual elimination of the former pulmonary pathogens, and second the emergence of antibiotic resistant strains of staphylococcus in recent years.

As was remarked earlier, in the present study all gram-positive organisms were tested for sensitivity to five antibiotics. Of eighty-two strains of staphylococci isolated from lung and/or heart's blood, only twenty-one were sensitive to all five; of these, fifteen were recovered from patients who did not receive antibiotics. It is thus obvious that terminal infection is caused for the most part by more or less resistant strains. Of our patients with pneumonia from whom staphylococci were recovered, antibiotics had been used in 63 per cent. In those yielding staphylococci but without pneumonia, the figure was 74 per cent. Of the seventeen patients whose heart's blood contained staphylococci, 62 per cent were under treatment. These over-all figures would seem to indicate that, insofar as the likelihood of terminal staphylococcal infection is concerned, it makes little difference whether the patient is receiving antibiotics or not. The next question is whether antibiotics did harm. A critical review of our patients dying, with staphylococcal pneumonia as an important contributory cause and in whom recovery might otherwise have occurred, yields not a single instance that might

TABLE III
ORGANISM IN LUNGS

	Pat	ients	Pari	ients
	Pn mc	ith eu- onia 10)	Pn mo	hout eu- onia
		10)	-	1
	No.	%	No.	%
A, Staphylococci				
Staphylococcus recovered	54	50	27	30
Growth abundant in	45	83	17	63
Pure cultures	10		1	
B, Pyocyaneus				
Pyocyaneus recovered	26	24	6	7
Growth abundant in	14	54	1	18
Pure cultures	6		1	
C, Proleus	E			
D 1	22	24	4.0	4.0
Proteus recovered	23	21	12	13
Growth abundant in	11	48	5	42
Pure cultures	2		0	
D, Klebsiella-Aerogo	enes			
Klebsiella-aerogenes recovered	27	25	19	21
Growth abundant in	16	60	9	48
Pure cultures	0		4	
E, E. Coli				
E. coli recovered	21	19	12	13
Growth abundant in	12	47	8	67
Pure cultures	1		4	
F, Enterococci				
Enterococci recovered	30	27	27	30
Growth abundant in	14	47	12	44
Pure cultures	0		0	
G, Alpha Streptocoo	ci			
Alpha streptococci recovered	11	10	19	21
Growth abundant in	7	64	14	74
Pure cultures	2		4	
H, Higher Bacteri	a			
Higher bacteria recovered	30*	27	18*	20
				72
Growth abundant in	12	40	13	0 4

^{* 83} per cent of these patients were given antibiotics.

be called "antibiotic-induced." In the total series there was one patient who received "prophylactic" broad-spectrum antibiotics during the postoperative period and died of a fulminating staphylococcal enterocolitis. One presumes (without absolute assurance) that this death was due to the antibiotics. This is the only example, insofar as the staphylococcus is concerned, that antibiotics appeared to harm the individual patient. On the other hand, they did not seem to do any particular good.

At this point reference might be made to the association of the staphylococcus with influenza. In nine of our patients dying during the month of October 1957, Asian influenza virus was recovered from the lungs. One of these patients died of gastrointestinal hemorrhage. The other eight were believed to have died of influenzal pneumonia. In four of these patients the lungs showed a heavy growth of staphylococci, twice in association with a positive heart's blood culture. None of these patients had received effective antibiotic therapy prior to death. In two patients the staphylococcus seemed to have caused death, as the clinical course was prolonged and abscess formation was found at autopsy. The other two were fulminating cases, and it is impossible to say that death might not have occurred as a result of viral infection alone, although one is sure that the uninhibited growth of staphylococci was not helpful to the patient. Three of the four strains of staphylococci recovered from these patients were similar. All were resistant to penicillin but sensitive to the other four antibiotics. The phage type was 52A79.

In the four patients from whom staphylococci were not recovered, the bacteriologic findings were inconsequential. All of these had been thoroughly treated with antibiotics before death

Pseudomonas Aeruginosa (Pyocyaneus). Attention will now be directed to some of the gramnegative rods and the role they appear to be playing in bronchopneumonia. There are abundant references to Friedländer's bacillus in the literature as a rare cause of pneumonia, but little comment as to the others. With regard to pyocyaneus, Piringer and Pingera [3] reported studies made in Germany in 1940 of the lungs of 150 patients who had been autopsied. These investigators do not mention pyocyaneus in the list of organisms recovered. In 1934 Burn [4] made bacteriologic studies of 121 lungs and

found pyocyaneus in 5 per cent of them. This is a figure which is markedly lower than ours, as can be seen in Table III (section B).

The figures in Table III (section B) indicate that pyocyaneus was recovered four times as often in lungs from patients with pneumonia as in those without pneumonia, and the finding of an abundant growth was almost always associated with pneumonia. There would seem to be little doubt, therefore, that pyocyaneus has indeed become a pulmonary pathogen. That this is due to suppression of the ordinary flora by antibiotics seems equally clear, for 93 per cent of the patients showing abundant growth of this organism were receiving antibiotics at the time of death. This terminal invasion, antibioticinduced, with pyocyaneus can take place with astonishing speed, as is indicated by the following case report:

Case 91. A seventy year old Negro man, comatose and in extremis, was admitted with signs of meningitis, pneumonia and a septic knee joint. Pure cultures of type 22 pneumococcus were obtained from spinal fluid, blood, and pus from the knee. Type 22 pneumococcus was also cultured from the nose and throat. He died twenty-one hours after admission, having received during that time 27 million units of penicillin, together with chloramphenicol and sulfadiazine.

At autopsy no pneumococci could be recovered despite heroic efforts. However, pyocyaneus was cultivated from spinal fluid, trachea, lung, and pus from the knee joint.

This case is probably an exceptional one, but it does indicate what can happen. When found in the lungs, the association of pyocyaneus with the histologic finding of pneumonia is very striking. And yet it would appear rarely to be the sole bacteriological cause. In our list of cases in which pneumonia was an important factor in the death of the patient, pyocyaneus could only be incriminated twice as the sole bacterial agent. One must conclude, therefore, that it is less effective as a pulmonary pathogen than staphylococcus.

Pyocyaneus was infrequently recovered from the heart's blood, being present in only 12 per cent of the positive cultures. (In all but one of these, pneumonia was present.) This is puzzling, since it is generally quite resistant to antibiotics. All gram-negative bacilli were tested against three antibiotics: streptomycin, tetracycline and chloramphenicol. Among thirty strains of pyocyaneus tested, only one was sensitive to all three.

Proteus. Organisms of this group, several of which are considered pathogenic, are usually thought of in connection with infections of the urinary and gastrointestinal tracts in man. There is little reference to them in the lungs. Burn [4] found proteus in 5 per cent of 121 lungs. As with respect to pyocyaneus, our figure is somewhat higher. The proteus "scoreboard" is given in Table III (section C).

These figures indicate that proteus was recovered more frequently from lungs of patients with pneumonia than those without, but the difference is less than twofold. However, there is surprisingly little difference in the incidence of abundant growths, despite the well known tendency of this organism to "swarm." As the sole bacterial cause of the pneumonia which precipitated a patient's death, proteus appears only once in our series. This patient was uremic and had received no antibiotics.

In sharp contrast to pyocyaneus, the finding of proteus in the lungs was not associated with antibiotic therapy. Of the twenty-three patients with pneumonia from whom proteus was recovered, only 52 per cent were receiving antibiotics—a figure appreciably lower than for the series as a whole (68 per cent). This is surprising in view of the fact that, like pyocyaneus, proteus tends to be resistant. Only two of twenty-seven strains were sensitive to all three antibiotics. This being the case, too, one might have expected to find proteus more often in heart's blood cultures. It was recovered only five times in fifty positive cultures. (Four of these five patients had pneumonia, and all were receiving antibiotic therapy.)

One may conclude that proteus is endowed with rather modest powers as a contributor to terminal pneumonia in man.

Klebsiella-Aerogenes. Klebsiella pneumoniae, or Friedländer's bacillus, has long been recognized as a rare cause of primary pneumonia in man, and reference to the clinical features of "Friedländer's pneumonia" is found in all the textbooks. Within recent years, however, it has been increasingly evident that it is impossible by the criteria of capsule formation and biochemical reactions to distinguish unequivocally between klebsiella and aerobacter aerogenes. In consequence, bacteriologists now refer to both groups of organisms as "klebsiella-aerogenes," and we have followed this procedure in our study.

Because of the well established reputation of

Friedländer's bacillus we had anticipated finding klebsiella-aerogenes playing a fairly important role as a pulmonary pathogen in our series. That this was not the case is shown in Table III (section D).

As can be seen, klebsiella-aerogenes was recovered almost as frequently from the lungs of normal subjects as from those with pneumonia; also there was little difference in the likelihood of an abundant growth being found in the two groups. Oddly enough, it was not recovered in pure culture from any of the lungs from patients with pneumonia.

On the other hand, on review of our cases in which pneumonia was considered a contributory cause of death, klebsiella-aerogenes was deemed the single important bacterial agent in two. Moreover, it was recovered from 28 per cent of our positive heart's blood cultures, being second in this regard only to the staphylococcus. This may seem a curious finding in view of the fact that organisms of this group have considerable antibiotic sensitivity, thirty-two of fifty-five of our strains being sensitive to all three test antibiotics. However, it was recovered only once in the presence of an effective antibiotic.

One of the two cases in which klebsiella-aerogenes was believed to have caused fatal pneumonia was of metastatic carcinoma. This patient did not receive antibiotics during the week prior to death. The other was an eighty-two year old postoperative patient who received chloramphenicol for the last ten days of life—and the strain of klebsiella-aerogenes was sensitive to this agent. It may be concluded that most organisms of this group do not have great capacity as pulmonary pathogens despite their frequent occurrence in the lungs.

E. Coli. Colon bacilli are found, often in mixed cultures, in inflammatory lesions related to the gut, and frequently give rise to infections of the urinary tract. Bacteremia may accompany severe infections of the urinary tract, but metastatic foci are uncommon. As causative agents of terminal pneumonia they have received little attention in the literature. Our experiences with E. coli are summarized in Table III (section E).

The tabulation indicates that E. coli was recovered somewhat more frequently when pneumonia was present. On the other hand, the likelihood of finding an abundant growth was actually greater in the absence of pneumonia. E. coli was also recovered in 24 per cent of our

Table IV

Strain	No
Candida albicans	37
Candida tropicalis	8
Candida krusei	
Candida parapsilosis	2
Trichosporon	
Rhodetorula	
Nocardia asteroides	1
Aspergillus	10

positive heart's blood cultures despite the fact that it is relatively susceptible to antibiotics, twenty-five of thirty-nine strains being sensitive to all three against which they were tested (only thrice, however, in the presence of an effective agent). In one case in which pneumonia was considered to be an important contributing cause of death it seemed to be due primarily to infection with E. coli. This patient had a malignant lymphoma of the brain, and died twelve days following a craniotomy. She had received no antibiotics for five days prior to death.

A review of the E. coli "scoreboard" implies that its significance in terminal pneumonia is about the same as that of proteus and klebsiella-aerogenes.

We shall now discuss briefly two gram-positive organisms which do not seem to be implicated in terminal pneumonia.

Enterococci. Table III (section F) indicates that enterococci were recovered more often from lungs of patients without pneumonia than from those with pneumonia, and the finding of abundant growths was approximately equal in the two groups. None of our patients appeared to have died of enterococcal pneumonia. So, despite the fact that enterococci are relatively resistant (only nineteen of seventy-nine strains were sensitive to all five antibiotics), they do not appear to be pulmonary pathogens. As might be expected, owing to their antibiotic resistance, they were encountered fairly frequently in the heart's blood (20 per cent of our positive cultures). The association of pneumonia with the finding of the organism in the blood was the same as the incidence of pneumonia for the series as a whole. The majority of the strains of enterococci isolated were hemolytic.

Alpha Streptococci. The "scoreboard" for

TABLE V
INSTANCES IN WHICH A SINGLE ORGANISM WAS
CONSIDERED RESPONSIBLE FOR TERMINAL
PNEUMONIA

	Or	ga	117	is	n	1								No
Staphylococcus														17
Pyocyaneus														2
Proteus														1
Klebsiella-aerogenes														2
E. coli														1
Higher bacteria														3

alpha streptococci is given in Table III (section G). These data show a negative correlation of alpha streptococci with terminal pneumonia. They were never regarded as a cause of death. In view of their uniform sensitivity to antibiotics (all twenty-six of our strains were sensitive to five antibiotics), it is not surprising that they were infrequently found in the heart's blood (4 per cent of our positive cultures).

Higher Bacteria. Yeasts and moulds have received increasing attention as potential pathogens in recent years. This is partly as a result of the development of mycology as a diagnostic aid, and partly due to the phenomenon of superinfection with yeasts in patients treated with antibiotics, or as a complication of steroid therapy. Our experiences with higher bacteria are indicated in Table III (section H).

These figures show relatively little difference between patients with pneumonia and those without with respect to the finding of higher bacteria. Moreover, abundant growths were encountered nearly twice as often in lungs from patients without pneumonia. Thus, the "scoreboard," while it indicates a relationship with antibiotic therapy, does little to incriminate the higher bacteria in terminal pneumonia. However, three patients seem to have died of pneumonia caused by these organisms. It is unlikely that any of these three patients would have survived their underlying illnesses. One may conclude that higher bacteria may cause pneumonia, that this is probably abetted by antibiotic and steroid therapy, but that the event occurs only with grave underlying disease.

The pathogenic higher bacteria recovered are listed in Table IV. Incidentally, yeasts were found in 6 per cent of our positive heart's blood cultures. In each instance the patient was receiving antibiotics.

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Table VI PNEUMONIA WITH ABUNDANT BACTERIAL GROWTH

Organism	Antibiotic Therapy (%
Staphylococcus	66
Pyocyaneus	93
Proteus	45
Klebsiella-aerogenes	69
E. coli	50
Higher bacteria	75

In Table v is given a recapitulation of the number of instances in which a single one of the aforementioned organisms was believed to be the probable cause of fatal terminal pneumonia.

Relationship to Antibiotics. Some indication of the relationship of antibiotics to the finding of certain pathogenic microorganisms in the lungs is given in Table VI. Here are considered all the patients in whom pneumonia was found at autopsy, and whose lungs yielded an abundant growth of the various bacteria in question, in terms of antibiotic therapy. In evaluating this table one must bear in mind that, in the entire series, 68 per cent of the patients were under treatment at the time of death.

In this tabulation, one organism—pyocyaneus—stands out. Nearly all the patients from whom this microbe was recovered in large amounts were receiving antibiotic therapy, and the infection may rightly be termed "antibiotic-induced." Other organisms in relation to which the percentage on antibiotics was higher than the average were the higher bacteria. Antibiotics seemed to have no influence on the finding of staphylococci and klebsiella-aerogenes, whereas they seemed to act as somewhat of a protection against proteus and E. coli.

TRACHEAL CULTURES

Reference has been made in the section on "Materials and Methods" to the fact that cultures were made from a tracheal swab as a routine in all cases. Our findings were in accord with those reported by Smillie and Duerschner in that these swabs always showed bacterial growth, and that if an organism was recovered from the lungs it was almost invariably present in the trachea as well. The reverse, of course, was not true, and if one expresses in terms of percentage the likelihood of an organism found in the trachea being present in the lungs as well,

TABLE VII INVASION RATES

Organism							
Staphylococcus aureus	7						
Pyocyaneus							
Proteus							
Klebsiella-aerogenes	6						
E. coli							
Enterococci	6						
Alpha streptococci	4						
Neisseria							
Staphylococcus albus							

this might be termed the "invasion rate." These invasion rates are given in Table VII.

These figures merely indicate that the organisms which have been considered potential pathogens show a higher invasion rate than the nonpathogens, with the single exception of the enterococcus, which had a surprisingly high rate.

TYPES OF DISEASE ASSOCIATED WITH PNEUMONIA

It seemed of interest to study bacteriological findings and the incidence of terminal pneumonia in relation to underlying disease. A review of our 201 cases indicated that in 145 of them death could be principally ascribed to one of six major causes: malignant disease, hepatic failure, uremia, cerebral disease (apoplexy, brain tumor, brain surgery), congestive heart failure and myocardial infarction. The incidence of terminal pneumonia in each of these is given in Table VIII.

The likelihood of finding terminal pneumonia is by far the least in myocardial infarction, which is not surprising in view of the fact that in this category death is frequently sudden. The percentage of patients showing pneumonia is

TABLE VIII
TERMINAL PNEUMONIA IN DIFFERENT DISEASE GROUPS

Disease Group	Cases (no.)	Pneumonia (%)
Malignancy	42	55
Liver	23	48
Kidney	20	65
Brain	28	64
Congestive heart failure	19	58
Coronary	13	8

Table ix bacteria in patients without pneumonia

Disease Group	Cases (no.)	Growth Abundant (%)	Growth Scanty (%)	Sterile
Malignancy	19	63	26	10
Liver	12	92		8
Kidney	7	57	29	14
Brain	8	13	25	63
Congestive heart				
failure	8	25	37	37
Coronary	11	18	9	73

almost the same in malignant disease and heart failure as in the series as a whole (55 per cent). It is a little higher in patients with uremia and in those with cerebral disease. We were somewhat surprised, however, to find that the incidence of terminal pneumonia in cholemia was a trifle lower than in the series as a whole, particularly as it was our impression that the patients with liver disease were exceptionally "buggy." It therefore seemed of interest to extract from our data the bacteriological findings in patients without pneumonia. It may be pointed out here that for the entire group of ninety-one patients without pneumonia, bacteria were found in two-thirds of the lungs, and this figure was precisely the same whether antibiotics were used or not. Finally, it may be stated that we have no evidence that the presence of bacteria in the lungs is a result of postmortem invasion, that is to say, the finding of bacteria in the lungs of patients without pneumonia was quite unrelated to the time elapsing between death and autopsy. A similar belief has been expressed by others [4-8].

The most striking feature of Table IX is that, while slightly more than half the patients dying of liver failure did not have pneumonia, their lungs were swarming with bacteria. It is possible to imagine that the cholemic patient is an excellent culture medium, and at the same time has a diminished capacity for inflammatory response. The reverse seems to be true of patients with cerebral disease; more than the average of these have terminal pneumonia, but in those who do not the lungs are remarkably clean bacteriologically. One can infer that a patient dying in coma of cerebral origin is likely to have pneumonia if organisms reach his

Table ,x disease groups, pneumonia and antibiotics

Disease Groups	Cases (no.) Anti-		ases biotics			No Pneumonia		
	(110.)	(%)	%	%	%	%		
Malignancy	42	52	55	42 Ab*	45	66 Ab		
Liver	23	78	48	82 Ab	52	75 Ab		
Kidney	20	70	65	69 Ab	35	71 Ab		
Brain	28	79	64	78 Ab	36	80 Ab		
ure	19	68	58	91 Ab	42	38 Ab		
Ceronary	13	54	8	100 Ab	92	50 Ab		

*Ab refers to percentage of those receiving antibiotics at time of death.

lungs. Lastly, the high incidence of sterile lungs in the patients with myocardial infarcts is also noteworthy.

The relationship of antibiotics to the finding of terminal pneumonia in the various disease groups is indicated in Table x. At first glance this may seem formidably complicated, but actually the table is quite simple. In the first column is given the number of cases in each disease group and in the second is given the percentage of these receiving antibiotics at the time of death ("Ab" being the symbol). The third and fourth columns, respectively, show the percentages with and without pneumonia, and in parentheses are given the percentages of these receiving antibiotics. In considering the figures it may be borne in mind that, in our total of 201 cases, pneumonia was found in 55 per cent, and 68 per cent of all the patients received antibiotics.

It will be observed that the pneumonia rate in malignant disease is precisely the same as in the series as a whole despite the fact that antibiotics were given more sparingly than in other conditions. However, as antibiotics were used more commonly in those without pneumonia they may, perhaps, have had some prophylactic effect in this single disease group. This is in striking contrast to the cases of congestive heart failure in which the pneumonia rate was almost exactly the same, but the complication occurred far more frequently with antibiotic treatment than without it. The pneumonia rate was higher than average in the uremic patients and in those with cerebral disease, but appeared to be completely uninfluenced by antibiotics. Reference has already been made to the lower than average pneumonia rate in patients dying of hepatic failure. While this was associated with

Table XI
PNEUMONIA CONTRIBUTORY TO DEATH IN DIFFERENT
DISEASES

DISEASES						
Disease Group						
Malignancy	3.3					
Liver	17					
Kidney	30					
Brain	29					
Congestive heart failure	32					
Coronary	0					

free use of antibiotics in the group as a whole, they did not seem to be playing much of a role as they were administered slightly more often to patients with pneumonia than to those without.

The figures cited relate to all pneumonias irrespective of whether they were an incidental autopsy finding or contributory to the death of the patient. The likelihood of pneumonia occurring as the cause of death or an important contributor thereto in the different disease groups is given in Table x1.

These figures indicate that in roughly a third of four major disease groups terminal pneumonia is an important factor in the death of the patient. The incidence would appear to be significantly lower in patients dying of liver failure, and, of course, in coronary occlusion. This re-emphasizes a point made earlier, that, despite the presence of masses of bacteria, the patient with cholemia has a limited capacity for inflammatory response.

HEART'S BLOOD CULTURES

For one reason or another, heart's blood was not obtained in all our cases. Only 141 specimens were actually cultured, and of these, fifty showed growth of one or more organisms. That these positive cultures had some slight significance in relation to pneumonia is indicated by the fact that positive cultures were found about 25 per cent more frequently in its presence than in its absence. Of more interest to us was the relation of our disease groups to the incidence of bacteremia. In Table xII the percentage of patients with positive heart's blood cultures is given in the first column, and the second column shows the percentage of these receiving antibiotics.

Once again the cholemic patient appears to be the best culture medium despite the free use of antibiotics. It is not surprising that bacteremia

TABLE XII
BACTEREMIA AND DISEASE GROUPS

Disease Group	Bacteremic (%)	Ab (%)*
Malignancy	50	55
Liver	66	70
Kidney	50	57
Brain	28	60
Congestive heart failure	0	
Coronary	8	100

* Percentage of those receiving antibiotics at time of death.

should be so rare in coronary occlusion in which death tends to be sudden, but the failure to find a single positive culture in congestive failure, in which death is usually lingering, is impossible to explain. It cannot be ascribed to antibiotics which were administered to precisely the same percentage of this disease group as to the series as a whole (Table x). The low rate in the patients with cerebral disease also is noteworthy. It might be added that in this disease group the finding of bacteremia was invariably associated with pneumonia.

The retrospective clinical significance of bacteremia at autopsy does not appear to be very great. A survey of our entire series discloses but one case in which death seemed to occur as a direct result of sepsis in a patient who might otherwise have lived a little longer. This patient had postnecrotic cirrhosis of the liver and was admitted in moderate cholemia. Mounting fever, nausea, vomiting and abdominal tenderness developed. A blood culture on the last day of life showed E. coli, and the same organism was obtained at autopsy. The administration of antibiotics subsequently shown to be effective against this organism was started only a few hours before death. The clinical data in this case suggest that the prognosis on admission was very poor in regard to the underlying disease; death, however, was undoubtedly precipitated by sepsis.

It would seem logical to suppose that antibiotics administered prior to death would have a determining influence on the type of flora recovered from the heart's blood at autopsy. This is true in the main, but their effect is not completely suppressive. Of sixty-eight strains of bacteria recovered from the heart's blood, thirteen, or nearly one-fifth, were shown to be sensitive to an antibiotic with which the patient was being treated at the time. DO ANTIBIOTICS CAUSE HARM?

It is widely stated in current medical writing that the indiscriminate use of antibiotics is harmful to the patient. The only question one is entitled to ask oneself in a study of the kind we have made is, "How often is it disastrous?" That is to say, in how many instances were antibiotics, needlessly applied, responsible for the death of the patient? A review of our entire series produces two. Both were postoperative patients who should have survived. Each received "prophylactic antibiotics," each died of pseudomembranous enterocolitis. One was associated with staphylococcus (referred to earlier), and the other with klebsiella-aerogenes and enterococci which were recovered from the gut. Pseudomembranous enterocolitis was well known before the antibiotic era; the incidence now, however, would appear to be considerably greater than formerly, and this increase is currently deemed to be attributable to antibiotics.

The fact that we ascribe only two deaths to antibiotics may surprise any reader who remembers that in an earlier section we clearly related pyocyaneus pneumonia to antibiotics and stated that two of our patients had died of this condition. But in each of these the antibiotic therapy was absolutely indicated; one was an acute staphylococcal endocarditis and the other an elderly patient with Parkinson's disease who had pneumococcal pneumonia on admission. The same statement may be made in regard to other patients receiving antibiotics who died of pneumonia due to resistant organisms or higher bacteria-that the antibiotics were really indicated, or that the underlying disease was lethal anyway. This does not imply that we condone the needless and indiscriminate use of antibiotics. Every clinician is aware of the unpleasant consequences of such use, such as the appearance of resistant staphylococcal infections in hospitals, unnecessary (and on very rare occasions fatal) allergic reactions, stubborn moniliasis, prolonged gastrointestinal dysfunction and interference with bacteriological diagnosis. We merely wish to state that in 201 cases studied at autopsy in a large general hospital, needless use of antibiotics was only twice responsible for the death of a patient who might otherwise have survived.

DO ANTIBIOTICS DO GOOD?

It is quite obvious that this question cannot be answered by a study of patients all of whom

are dead. Every clinician whose memory goes back to the pre-antibiotic era is aware of how profoundly, how miraculously, the outlook in regard to almost all infections has been altered. The fact that the gross figures in respect to terminal pneumonia seem to have changed so little does not rule out the strong likelihood that many of our 201 patients lived longer owing to antibiotics than they would have thirty years ago. Patients will inevitably continue to die of cancer, uremia and cerebral apoplexy, as they have done in the past. Terminal pneumonia will continue to be found, as in the past, but caused nowadays by less efficient pulmonary pathogens than formerly. And that even in these irreversibly lethal conditions antibiotics continue to work is indicated by one final statistic: A review of our cases in which pneumonia was contributory to death vields only five instances in which the patient was receiving an antibiotic to which his organism was sensitive.

SUMMARY

This bacteriological study of 201 autopsy reports was designed to throw some light on what is being accomplished, with particular reference to pneumonia, by the widespread use of antibiotics as currently practiced. For purposes of comparison, we had the past records in our own Department of Pathology, and the reports of similar studies performed before the introduction of these relatively new therapeutic agents. Certain obvious conclusions were immediately apparent: "Old-fashioned" lobar pneumonia as a cause of death has almost entirely disappeared, and the organisms that were formerly considered of greatest significance in terminal pneumonia, all of which are uniformly sensitive to antibiotics and do not tend to acquire resistance, have likewise withdrawn from the scene. These three are the pneumococcus, the group A hemolytic streptococcus and H. influenzae. Their place has been taken by the staphylococcus, which has become relatively more prominent than formerly, and several gram-negative bacilli.

As the single bacterial incitant of pneumonia contributory to the death of the patient, staphylococci were incriminated nearly three times as often as all the gram-negative bacilli combined. However, when one considers total numbers (seventeen of 201), one does not get the impression of an epidemic out of control. The finding of staphylococci and pneumonia at autopsy

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appeared to be uninfluenced by antibiotic treatment prior to death. Most of the strains recovered were more or less resistant to antibiotics, but a review of all our cases yielded not a single instance in which a fatal staphylococcal pneumonia appeared to have been induced by needless antibiotic therapy in a patient who might otherwise have recovered. Undoubtedly terminal staphylococcal pneumonia is a prominent feature of the clinical scene today, but the finding of staphylococci in nearly one-third of normal lungs (more often than not in abundant growth) suggests that this organism is a less specialized pulmonary pathogen than the pneumococcus.

Pyocyaneus was next in importance to staphylococcus in relation to terminal pneumonia, and was found almost exclusively in patients receiving antibiotics. It was considered only twice, however, as the sole bacterial cause of pneumonia contributory to the death of the patient. Thus the organism would seem to have limited potentialities as a pulmonary pathogen. Pyocyaneus pneumonia appears to be a product of the antibiotic era, yet we did not find a single fatal case of this disease in which antibiotics had been needlessly employed in a patient who might otherwise have survived.

Klebsiella-aerogenes appeared to have about the same significance in terminal pneumonia as pyocyaneus. Infection with this organism, however, seemed unrelated to antibiotics. Terminal pneumonias associated with proteus or E. coli occurred more commonly in patients not receiving antibiotics.

We have also reported the bacteriological and histologic findings in six major disease groups which accounted for 145 of our 201 cases. As might be expected, the incidence of pneumonia and of positive bacteriological cultures is extremely low in patients dying of coronary insufficiency. The most noteworthy finding in the other five disease groups, all of them chronic, was that the patient with cholemia appeared to be an excellent culture medium but with a limited capacity for inflammatory response.

There were two cases of pseudomembranous enterocolitis in our series. Both these patients had received "prophylactic" antibiotics during the postoperative period, and we have assumed that death in each case was the result of unnecessary use of antibiotics. These are the only two instances of such an event in a patient who might otherwise have survived. As there is

obviously no way of determining in a series of patients who have died how often antibiotics save life, the vexed question of the advisability of their widespread use cannot be answered from our data. We may simply restate our opinion that antibiotics have modified the pattern rather than the incidence of terminal pneumonia.

Finally, we should like to give a word of comfort to the ardent therapeutist who wishes to fight the longest possible rear-guard action in a hopeless cause. We have considered in some detail six organisms which seem to have taken over the role of terminal pulmonary pathogens. In twenty-six cases pneumonia contributory to the death of the patient appeared to be due to a single one of these bacteria, and in only five instances was the patient receiving an antibiotic to which that particular organism was sensitive. One concludes that the right antibiotics still have efficacy even at the end stage of chronic disease.

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REFERENCES

- SMILLIE, W. G. and DUERSCHNER, D. R. The epidemiology of terminal bronchopneumonia. I. The significance of post-mortem cultures in determination of the etiology of terminal pneumonia. Am. J. Hygiene, 45: 1, 1947.
- SMILLIE, W. G. and DUERSCHNER, D. R. The epidemiology of terminal bronchopneumonia. II. The selectivity of nasopharyngeal bacteria in invasion of the lungs. Am. J. Hygiene, 45: 13, 1947.
- PIRINGER, W. and PINGERA, L. Untersuchungen über die Keimflora der tieferen Luftwege der Leiche. Biol. Abstracts, 162: 1603, 1942.
- Burn, C. G. Post-mortem bacteriology. J. Inf. Dis., 54: 395, 1934.
- Burn, C. G. Experimental studies of post-mortem bacterial invasion in animals. J. Inf. Dis., 54: 388, 1934.
- GIORDANO, A. S. and BARNES, A. R. Studies in post-mortem bacteriology: Value and importance of cultures made post-mortem. J. Lab. & Clin. Med., 7: 538, 1922.
- 7. Hunt, H. F., Barrow, E., Thompson, L. and Waldron, G. A bacteriologic study of five hundred and sixty-seven post-mortem examinations. *J. Lab. & Clin. Med.*, 14: 907, 1929.
- PUTNOCKY, J. Results of bacteriological studies of 400 necropsies. Am. J. Clin. Path., 7: 275, 1937.

Pulmonary Disease in Adults Associated with Unclassified Mycobacteria*

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NCLASSIFIED mycobacteria, both pigmented and non-pigmented types, have been isolated from human sources with increasing frequency since first reported [1-11]. However, clinical and bacteriologic interest in these organisms did not become widespread until the past six years. Since 1953 studies by Runyon and many others [12-22] have established some consistent bacteriologic differences between unclassified mycobacteria, human and bovine tubercle bacilli, isoniazid-resistant catalasenegative mutants of Mycobacteriun tuberculosis, and some of the saprophytic mycobacteria. These workers have labeled the unclassified mycobacteria thus far identified as groups I, II, III and IV, more popularly known as the photochromogens, scotochromogens, non-chromogens (often called Battey type), and "rapid growers," respectively. Although these names are obviously only descriptive terms, the bacteriologic identification of the photochromogens and scotochromogens has been fairly consistent in different laboratories. The characteristics of the nonchromogens and the rapid growers are much more variable and probably these two groups actually include several different strains.

Following this tentative bacteriologic classification, there has been an increasing number of case reports of pulmonary and other diseases in man associated with the isolation of unclassified mycobacteria from various secretions and tissues [22–34]. It is apparent from these reports that the incidence of the different unclassified mycobacterial strains varies with the locality. It has also been noted that some strains are more often associated with human disease than others. The majority of the isolations of unclassified mycobacteria reported have been from patients with

pulmonary disease, but a few case reports of cervical adenitis in children [35], osteomyelitis [36] and disease in other organs [29] have also implicated these organisms as the possible pathogens.

The picture of unclassified mycobacterial pulmonary infections in adults remains variable and often confusing. As more cases are reported, however, certain clinical patterns are beginning to emerge. At the same time the unanswered questions regarding the epidemiology, pathology and treatment of this disease have become more obvious

In an effort to clarify further some of these problems the records of all cases of pulmonary disease associated with the isolation of unclassified mycobacteria observed on the adult tuberculosis division of Parkland Memorial Hospital in the past three years have been reviewed in detail. The total number of cases reviewed represented about 1 per cent of all admissions to the tuberculosis division during the time of the study.

METHODS

Each patient observed had a minimum of six sputum cultures for acid-fast organisms, as well as routine sputum cultures for pyogens and fungi, on admission to the hospital. This was followed by a minimum of one acid-fast culture every four weeks until discharge from the hospital; thereafter by cultures every three months during the complete observation period. In addition, patients who had undergone resection had a minimum of three postoperative acid-fast sputum cultures during the first three weeks following surgery. All patients with positive reaction to fungal skin tests had an additional six sputum cultures for fungi.

Sputum, after concentration with trisodium phos-

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TABLE 1 IDENTIFICATION SCHEME

Organism	Colony and Rate of Growth	Liquid Media	Plain Agar	Sabouraud's	Neutral Red	INH Resistant	Cata- lase	Cord- ing	Morphology
Myocobacterium tuberculosis	Cream color, dry, brittle, bread crumb appearance; 3 to 6 weeks	Granular, difficult to suspend	-	-	Red	-	+	Tight	Slender, delicate curved rods, small granules
Group 1, photo- chromogen	Smooth or rough cream color in dark, turning yel- low-orange on exposure to light; 7 to 14 days	Suspends in swirling pattern	±	Scant, turns yellow	Brick-dust color	Growth in low concen- trations	++++	Loose	Longer, thicker, straight, large granules; may appear beaded
Group 11, scoto- chromogen	Smooth yellow-orange col- onics in dark or light; 7 to 14 days		+	-	May be brick- dust or negative	Growth in low concen- trations	++++	-	Longer, thicker, straight, large granules; may be beaded
Group iii, non- chromogen	White-cream, smooth (oc- casionally rough) colonies in dark or light; 2 to 4 weeks		±	-	Negative	Growth in low concen- trations	++	_	Very pleomorphic coccoid to bacil- lary heavy gran- ules

phate, was inoculated on Petragnani and Löwenstein-Jensen media, and incubated at 37°c. for eight weeks before being discarded as negative for acid-fast organisms. One tube was wrapped with aluminum foil to exclude light, and observed at regular intervals for development of, or change in, pigmentation of colonies. If positive cultures were observed to have pigmentation or colonial and cellular morphology suggestive of mycobacteria other than M. tuberculosis, they were then subjected to the scheme shown in Table 1 for further identification. This scheme was developed by Dr. Ruth Guy of the Department of Microbiology for routine use in our laboratory, and utilized the tests that were the most practical and consistently reproducible in a routine hospital laboratory. Konno's niacin test [37] probably would be a valuable addition to this identification scheme.

Lung tissue specimens, approximately 2 by 2 cm. by 4 mm. in size, were macerated in a Virtis Homogenizer for thirty minutes. Five ml. of normal saline and a few grains of sterile sand were added to produce a liquid homogenate. The homogenized suspension was inoculated on Middlebrook's 7H₄ agar, Pfizer's medium, charcoal agar, Löwenstein-Jensen medium and Dubos broth, and incubated at 37°c. All cultures were held for one year before being discarded as negative. All positive cultures were further identified by the same scheme used for identification of positive sputum cultures, as outlined in Table 1.

Drug sensitivity studies were performed on Löwenstein-Jensen medium with the following drug concentrations incorporated: 1 and 5 μ g. isonicotinic acid hydrazide; 10 and 40, or 100, μ g. streptomycin; 10 and 100 μ g. para-aminosalicylic acid; 10 and 100 μ g. viomycin sulfate. The amount or absence of growth on the media containing drugs was compared to growth on control non-drug containing media in the usual

manner. The results were reported as follows: sensitive, to all concentrations tested; partially resistant, to indicate resistance to low concentrations only; and resistant, to all concentrations tested.

OBSERVATIONS AND COMMENTS

The pulmonary infections associated with isolation of unclassified mycobacteria observed in our hospital have, in general, fallen into three groups. The data on each group will be presented separately, in order to illustrate more clearly the unanswered questions regarding pulmonary mycobacterial disease.

Group I. No Active Pulmonary Infection. This group included all patients who had only one random sputum culture positive for unclassified mycobacteria, usually scotochromogens, but who had either no roentgenographic evidence of disease or fibrotic areas which had been demonstrated to be unchanged in serial roentgenograms over a number of years. These patients were either completely asymptomatic, or had symptoms of other diseases which led to the original examination of the sputum and roentgenogram. Some of them had chronic respiratory diseases such as bronchiectasis, asthma or obstructive emphysema, but had neither new symptoms nor new lesions evident on the roentgenogram at the time the single positive sputum culture was obtained. Since no clinical significance could be attached to the single isolation of scotochromogenic mycobacteria in this group of patients, they were excluded from this study and will not be discussed further.

Group II. Mixed Pulmonary Infections. The second group included fourteen patients with mixed pulmonary infections, in whom one or more sputum cultures were positive for both M. tuberculosis and one of the unclassified mycobacterial strains. Three patients had sputum cultures positive for unclassified mycobacteria on admission, with isolation of M. tuberculosis later in the hospital course. The remaining eleven patients had unclassified mycobacteria isolated from sputum or tissue three to fortyeight months subsequent to the institution of continuous antituberculous drug therapy. None of the fourteen patients had a past history of tuberculosis nor treatment with the antituberculous drugs prior to their admission to the hospital.

The significance of the unclassified mycobacteria isolated subsequent to drug therapy in the eleven patients with typical pulmonary tuberculosis is not known. It may be that the unclassified mycobacteria were present at the time of admission, but not detected due to the larger numbers of tubercle bacilli present in the cultures. Or perhaps they were subsequent invaders of already damaged lung tissue. As pointed out by Wolinsky [31], the unclassified mycobacteria have consistent differences from isoniazid-resistant catalase-negative mutants of M. tuberculosis; therefore, the appearance of these unclassified mycobacteria subsequent to drug therapy cannot be attributed to drug effect.

Three patients in whom sputum cultures were initially positive for one of the unclassified mycobacteria, but subsequently positive for M. tuberculosis also posed a problem. As these patients had contact with active tuberculosis after admission to the hospital, the question of hospital infection with M. tuberculosis arose. In 1948 Wenkle and his associates [39] showed that vaccination of guinea pigs with unclassified mycobacteria produced a definite but partial immunity; however, it did not protect the animals from subsequent development of tuberculosis following infection with massive doses of a virulent strain of human type tubercle bacilli. To date, our data on these mixed infections do not provide the answers to such questions, but do point out some necessary lines for further investigation of this problem.

The history, symptoms, skin tests and x-ray findings in the fourteen patients with mixed pulmonary infections were compatible with those found in pulmonary tuberculosis. Twelve

had far advanced disease, one moderately advanced and one minimal disease. In all four-teen patients both the tubercle bacilli and the unclassified mycobacteria isolated from the sputum, either initially or during therapy, were partially or completely resistant to one or more of the three major antituberculous drugs. However, the drug sensitivities for the two organisms isolated from any single patient were always different.

All the patients in this group were treated with various combinations of isoniazid, streptomycin and para-aminosalicylic acid. In addition four had resections for residual cavitary lesions. During treatment bacteriologic conversion of the sputum occurred in eleven of the fourteen patients of this group. However, 50 per cent of these conversions were noted only after six months or more of treatment. Sputum cultures remained positive in the remaining three patients of the group, despite continuous prolonged drug therapy.

Two of the patients with persistently positive sputum cultures died as a result of progressive destruction of the lungs after two and a half and six years of drug therapy, respectively. The third still had positive sputum cultures after eleven months of drug therapy when he died of a cerebrovascular accident.

The eleven patients whose sputum became bacteriologically negative during drug and/or surgical therapy showed the following results: two have persistent extensive disease bilaterally on roentgenogram but have attained clinical stability; one, after eleven months of drug therapy in the hospital, shows slight but definite improvement in clinical status and x-ray findings; another, after eleven months of drug therapy in the hospital, has shown increase of disease on roentgenogram and progressive disability from his pulmonary disease; four are postoperative and have remained clinically well with negative sputum and stable roentgenograms during follow-up observations ranging from five to thirty-six months; three did not finish treatment and their current status is unknown.

In general, the response to treatment in this group of patients with mixed pulmonary infections, with the exception of the four patients who had resections of cavitary lesions, was not good. The high incidence of far advanced disease and initial drug resistance in the group should be noted when reviewing the results.

Group III. Unclassified Mycobacterial Pulmonary

Infections. The etiology of the pulmonary disease observed in the remaining patients appeared to be more directly attributable to unclassified mycobacteria. This group consisted of twentyfive patients with demonstrable pulmonary lesions of sufficient extent on the roentgenogram to warrant hospitalization for diagnosis. In all cases photochromogenic or non-chromogenic (Battey type) mycobacteria were cultured, usually repeatedly, from sputum or tissue or both. No tubercle bacilli or other known pathogens were isolated at any time from this group of patients despite repeated efforts to do so.

General data: In this group of twenty-five patients with unclassified mycobacterial pulmonary infection 80 per cent were males. Of the total group 72 per cent were white and 28 per cent Negroes. Ages ranged from twenty to sixty-five years, with a preponderance of the patients between ages thirty and fifty. Most of these patients were working people of middle or low income group, and about half were whitecollar workers. There were no alcoholics, vagrants or extremely debilitated persons in the group. The percentage of this group who had other serious systemic diseases was no higher than was found in hospitalized tuberculosis patients. There was no history of previous tuberculosis or of previous treatment with antituberculous drugs in any of these twenty-five cases.

Epidemiologic data: All patients in the group had resided in Dallas County for three to thirtyfive years prior to the time of admission to the hospital, and the majority had previously lived only in the northeastern part of Texas.

Data on family history and household contacts were considered highly significant. Of the twenty-five patients, family history for tuberculosis was negative in twenty (80 per cent), and positive or questionable in only five patients (20 per cent). Of the five patients listed as having positive family history, the relatives thought to have tuberculosis were distant and were not household contacts, except in one instance. The history for marital tuberculosis in the twenty-two married patients was entirely negative. In no instance in this group was there any history of isolation of unclassified mycobacteria in other members of the patient's family.

Total number of household contacts for the twenty-five patients was eighty-four, of whom fifty-seven (68 per cent) were checked. Chest roentgenograms obtained on twenty of these "contacts" were completely within normal

limits. The reaction to a Mantoux skin test performed on the other thirty-seven contacts. was negative in thirty-four and positive in three. One of the contacts who had a positive reaction to the tuberculin skin test was a registered nurse who had been working in a hospital for many years. Another was a child who was known to have had a positive reaction to a tuberculin test for several years prior to the time the father was found to have mycobacterial disease.

These data on family history and household contacts of patients with mycobacterial disease. although limited in number, are quite similar to the data reported on a larger group of patients from the Battery State Hospital in Georgia. They contrast with a higher incidence of positive roentgenographic findings and skin test reactions in contacts of patients with typical tuberculosis [32]. The findings suggest that the patients with unclassified mycobacterial disease are less infectious than patients with typical tuberculosis, and raise the question of a mode of infection other than man-to-man transmission. Although the literature reports many sources from which these organisms have been isolated, no definitive source of origin of human pulmonary infection with unclassified mycobacteria has been established.

Clinical data: Clinical history on these twentyfive patients indicated that half of them were admitted to the hospital on the basis of a routine chest roentgenogram for employment purposes, or for diagnosis of disease in some organ other than the lung. This half of the group was essentially asymptomatic. The other half had respiratory symptoms that led to an x-ray examination and the hospital admission. Seven of this latter symptomatic group had acute but very mild symptoms for five days to four weeks only. Five had mild symptoms of two to seven months' duration; in only one instance was the illness both progressive and severe.

In the symptomatic group the most frequent presenting symptom was mild hemoptysis (eight patients). Other symptoms noted were rather non-specific and included malaise, low grade fever, mild cough occasionally productive, generalized aching and mild pleuritic pain (four

In general the symptoms tended to be similar but much milder than those found in a similar group of patients with tuberculosis. The very paucity of symptoms in conjunction with cavitary lesions seen on the roentgenogram was one of the clinical patterns that emerged from study of this group.

Some investigators have suggested that previously damaged lung tissue might be a prerequisite for establishment of unclassified mycobacterial infection. Consequently, the absence or presence of emphysema in this group was particularly noted. There was both bullous and obstructive emphysema in six (24 per cent) of the twenty-five patients, but only two of these had evidence of severe symptomatic emphysema. This is about the same percentage of emphysema found among our hospitalized tuberculosis patients. No other chronic pulmonary diseases were noted in this group.

Roentgenographic findings: Roentgenographic extent of pulmonary disease was classified according to the diagnostic standards of the National Tuberculosis Association (1955). There were two minimal lesions, sixteen moderately advanced, and seven far advanced. Twenty-one of the twenty-five patients (84 per cent) had cavitary lesions. About one-third had bilateral disease. The distribution of disease was similar to that seen in tuberculosis.

Comparison of the roentgenograms of these twenty-five patients, taken on admission, with a similar number of admission roentgenograms of tuberculosis patients, taken at random, showed that there were no absolute pathognomonic roentgenographic features. Nevertheless, certain features were noted which suggested the presence of unclassified mycobacterial disease. These features were: (1) a higher percentage of cavitation for the total extent of lung involvement than is usually found in tuberculous disease; (2) thinner walled cavities surrounded by more strandlike and less dense infiltration than is usually found surrounding a tuberculous cavity; (3) fewer nodules than in tuberculosis; and (4) less roentgenographic evidence of bronchogenic spread than in tuberculosis; more evidence of contiguous spread.

The non-cavitary lesions assumed pleomorphic forms and were the most deceptive. They ranged from lesions resembling abscess of the lungs to strand-like fibrosis without much hilar elevation.

There were enough exceptions to the generalities listed that in any given case the roentgenograms were certainly not diagnostic and frequently not even suggestive of unclassified mycobacterial disease.

Skin test data: Skin tests with tuberculin, made on admission, in the twenty-five patients yielded

eighteen (69 per cent) positive reactions, six (27 per cent) negative reactions, and one doubtful. These findings reveal a higher percentage of negative reactions to tuberculin than is usually found in a comparable group of tuberculous patients.

Follow-up skin tests were carried out from three to forty months after the institution of drug therapy in twenty-four of the twenty-five patients. Of the six who initially had negative reactions to tuberculin skin tests, five were still negative at time of retesting seven to twenty-six months later; while one had a positive reaction to the skin test for the first time seventeen months after the institution of drug therapy. More interesting were three patients who initially had a positive reaction but in whom the reaction to follow-up tests had become definitely negative when repeated twenty-four, thirty-six and forty months later, respectively.

Skin tests with photochromogenic, scotochromogenic and non-chromogenic antigens were performed concurrently with tuberculin retesting in the same twenty-four patients and also in a group of an approximately equal number of tuberculosis patients taken at random. The results are too limited to permit any conclusions, but it was noted that cross reactions occurred between the tuberculin and the photochromogenic antigens in about 80 per cent of both groups. In general, in both groups those with marked reactions to tuberculin also had marked reactions to the photochromogenic antigen. However, reactions to tuberculin in the typical tuberculosis patients were larger in size than the photochromogenic reactions. An absence of cross reactions among the three chromogenic antigens tested was also noted. Further investigation of skin test results with mycobacterial antigens is now being carried out in a larger series of patients in an effort to confirm or deny these preliminary findings.

Bacteriologic data: The organisms isolated from this group of patients with obvious pulmonary disease were photochromogens in eighteen (72 per cent), non-chromogens in five, and unidentified chromogens in two. The predominance of photochromogens observed in our patients has also been noted in other Texas centers [40]. It should be noted that no scotochromogens were isolated from this group of patients with active pulmonary disease.

Drug sensitivity studies, performed before the institution of drug therapy, were available in

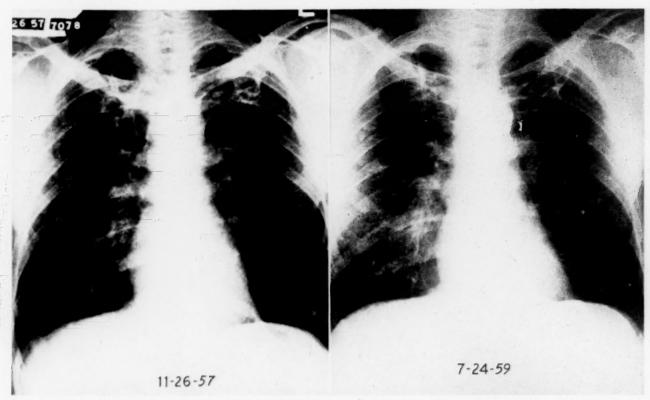


Fig. 1. Roentgenograms of patient C. T. demonstrated good improvement, although not complete clearing, during twenty months of continuous drug therapy. This patient's sputum converted after three months therapy; he was discharged from hospital after four months and has been working full time as a service station operator during the last twelve months of his drug treatment.

twenty-one of the twenty-five patients. Table II shows that they followed a pattern reported repeatedly in the literature [41–43]; that is, partial resistance to one or more drugs in most cases and slightly greater resistance to isoniazid than to streptomycin. It should also be noted that there was a higher percentage of initial drug-resistant organisms in the patients with unclassified mycobacterial disease than is usually found in a similar group of tuberculosis patients.

Most of the organisms tested showed some degree of resistance to para-aminosalicylic acid and viomycin sulfate.

Results of therapy: Various combinations of isoniazid, streptomycin and para-aminosalicylic acid, together with modified bed rest and supportive therapy when indicated, were used to treat twenty-four of the twenty-five patients. In addition to drug therapy, eleven patients had resections for residual cavities. In the remainder of the patients treated resection was not performed because of bilateral lesions, other diseases or evidence of continued definite improvement on drug therapy alone.

The results of therapy in the twenty-four

patients treated were determined by improvement visible on the roentgenogram and bacteriologic conversion of sputum. Clinical improvement could not be assessed adequately,

TABLE II
TWENTY-FIVE UNCLASSIFIED MYCOBACTERIAL CASES

Type	Number		soniaz	id	Stre	reptomyci			
Organism	of Cases	S	PR	R	S	PR	R		
Photochromo-							-		
gens	15	0	10	5	2	11	2		
Non-chromo-									
gens	5	1	1	3	2	1	2		
Unidentified	1	1	0	0	1	0	0		
Total—all									
strains	21	2	11	8	5	12	4		

Note: Drug sensitivity studies on initial cultures were not available in four cases and were performed prior to drug therapy in twenty-one cases.

S = Sensitive to all concentrations tested.

PR = Resistant to low concentrations only.

R = Resistant to all concentrations tested.

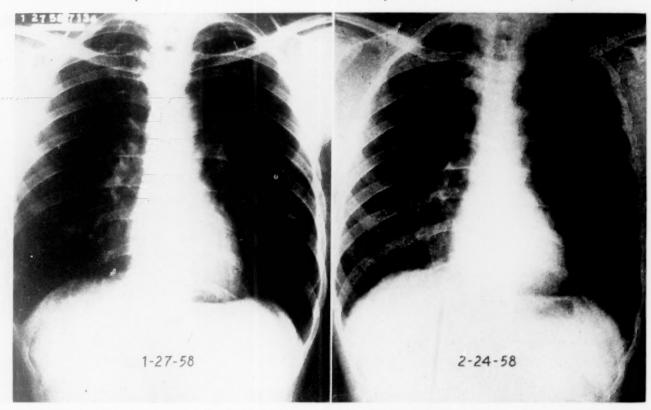


Fig. 2. Roentgenograms of patient E. G. illustrated complete resolution of cavitary lesion after three weeks of drug therapy. This patient's sputum on admission was positive on culture three times for photochromogens and negative for any other pathogen. The organisms cultured were resistant to isoniazid but sensitive to streptomycin. Treatment consisted of streptomycin, 2 gm. daily, and tetracycline for three weeks only.

except in the poor results, since most of the patients either had no symptoms or very mild symptoms which regressed rapidly within a week or so, often before drug therapy was begun.

Improvement observed on roentgenograms in the twenty-four patients treated was not marked on drug therapy alone. Progression of disease was observed in one patient, no change in six, slight to moderate improvement in fourteen, good improvement in two (Fig. 1), and complete resolution of the lesion without resection in one patient only (E. G.). (Fig. 2.) In general, the cavitary lesions remained unchanged or decreased slightly in size, and improvement noted on the roentgenogram was limited to moderate decrease in the exudative lesions surrounding the cavities. (Fig. 3.)

Bacteriologic conversion of sputum occurred in twenty-three (96 per cent) of the twenty-four patients treated, and over half of these conversions were noted in one to two months after the institution of drug therapy. All patients with resection had had conversion of sputum one month or more prior to surgery. The early conversion of sputum in many of the patients did not

necessarily correlate with improvement observed on the roentgenogram, drug sensitivity studies, or tissue negativity.

The one patient who did not have bacteriologic conversion of sputum was a thirty-eight year old white woman with persistently positive sputum cultures for photochromogens, increasing destruction of one lung as seen on the roentgenogram, progressive respiratory insufficiency, and a slowly progressive downhill course clinically during three years of continuous drug therapy. When lost to follow-up after three years of treatment, this patient was considered to be critically ill. (Fig. 4.)

Table III shows that from the eleven patients who had resections, tissue cultures for unclassified mycobacteria were positive in six and negative in four, although two of these were positive on tissue smear for atypical acid-fast organisms; one tissue culture was not available. No other known pathogens were cultured from these tissues. In all cases, the mycobacteria isolated from the tissue was the same strain that had been isolated from the patient's sputum initially. There was no good correlation between

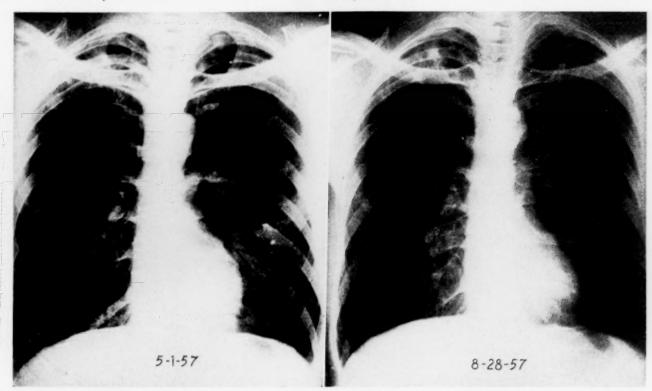


Fig. 3. Admission and preoperative roentgenograms of patient H. L. illustrated the most common roentgenographic response of cavitary lesions to drug therapy alone. This patient's sputum cultures were positive for photochromogens, which were partially resistant to isoniazid and sensitive to streptomycin. After five and a half months of treatment with both drugs, some clearing of exudation was visible on the roentgenogram but cavitation in the upper lobe of the right lung persisted. Segmental resection was performed and photochromogens were also recovered from tissue cultures. Patient has now remained well for twenty-two months since surgery.

tissue culture positivity and duration of drug therapy prior to resection. As might be expected, the patient who had only two months of drug therapy prior to resection had a positive tissue culture; so did the patient who had been treated for forty-three months prior to resection.

Duration of observations on these twenty-five patients with unclassified mycobacterial pulmonary infections ranged from four to forty-three months, and many are still receiving treatment. Therefore, not only is evaluation of long-term results of therapy impossible at this time but two other questions must also be answered before such a final evaluation can be made: progress of the disease without treatment, and percentage of relapses after therapy.

Regarding the natural course of the disease without treatment, the data from this group of twenty-five cases offer only two diametrically opposed examples: (1) One patient (Fig. 5) in the group, a forty-six year old white welder, with severe obstructive emphysema and sputum cultures positive for photochromogens, refused treatment and left the hospital against

medical advice to return to work. His chest roentgenogram on admission showed a small fibrocavitary lesion in the upper lobe of the left lung, which remained unchanged without treatment for seven and a half months. No follow-up sputum studies were available. Whether such a cavitary lesion will remain unchanged indefinitely is unknown. (2) Another patient (Fig. 6), a thirty-four year old Negro male laborer, had had serial roentgenograms taken in an outpatient clinic during the two years

Table III
ELEVEN RESECTED UNCLASSIFIED MYCOBACTERIAL CASES
Tissue Cultures After Drug Treatment (two to
forty-three months)

Type Organism	Positive	Negative	Not Available
Photochromogens	4	1	
Non-photochromogens	1	1	1
Unidentified	1	2	
Total	6	4	- 1

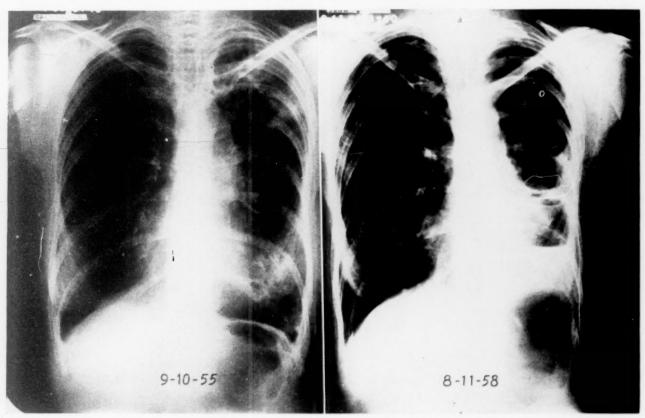


Fig. 4. Admission and three year follow-up roentgenograms of patient L. McW. whose disease progressed and whose sputum remained consistently positive for photochromogens during three years of continuous drug therapy.

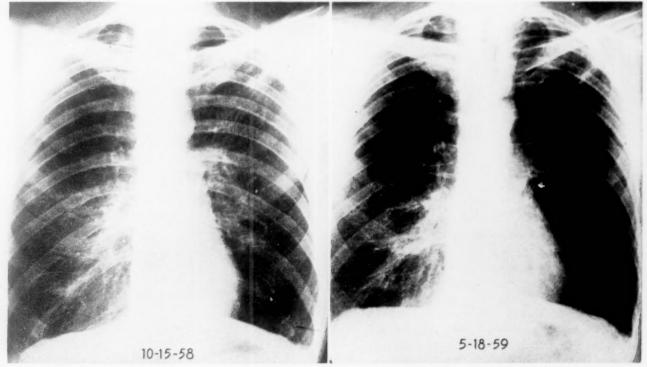


Fig. 5. Admission and follow-up roentgenograms of patient A. C. whose lesions remained unchanged during seven and a half month's observation period without treatment.

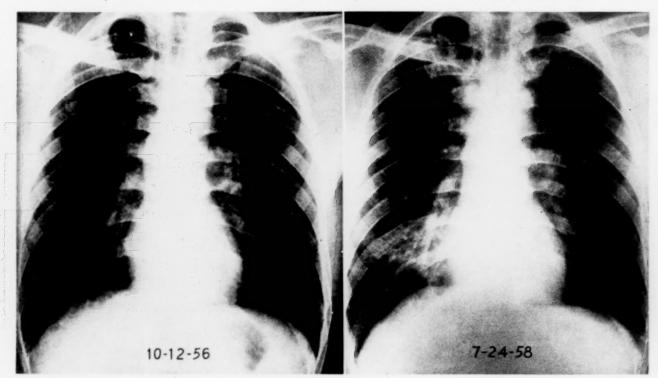


Fig. 6. Roentgenograms of patient E. C. obtained two years prior to hospital admission, and at time of admission. They showed progression of small nodular lesion in right apex to cavitation and spread to the middle lobe of the right lung prior to onset of treatment.

prior to admission to the hospital for treatment of photochromogenic disease. These films showed a small nodule in the right apex progressing, during the subsequent two years without treatment, to a cavitary lesion and spread of disease to the middle lobe of the right lung.

Obviously, a large number of patients without treatment would have to be observed over a period of years before any conclusions regarding the natural history of mycobacterial pulmonary disease could be reached.

In one patient of this group of twenty-four patients treated, the sputum culture has become positive again without concomitant roentgenographic or clinical change, and in another changes have been observed on the roentgenogram without positive sputum culture. In the first patient, two positive sputum cultures were obtained six months postoperatively, fourteen months after the institution of drug therapy, and while the patient was still receiving continuous drug therapy. However, the sputum has remained negative eighteen months since that time and the patient has continued to work. In the second patient, changes were observed on the roentgenogram thirty-three months postoperatively, thirty-eight months af er the institution of drug therapy, and twelve months after complete cessation of drug therapy. However, these changes were not related to recurrent symptoms or positive sputum cultures, and a lung biopsy specimen has revealed granulomatous lesions which are sterile on culture.

Tissue pathology: The tissue specimens from the eleven patients with resections were reviewed by Dr. George J. Race of the Department of Pathology and some attempt was made to compare them with similar specimens from typical tuberculosis patients. The same type of caseous granulomatous lesions were present in both. Microscopically, there were perhaps some fine points of difference noted. There was a larger amount of non-ulcerating, non-caseous, non-specific endobronchitis, usually sub-mucosally, in the specimens from the patients with unclassified mycobacteria than in the specimens from tuberculous patients. The number of caseous lesions were no greater than in tuberculosis, but the unclassified mycobacterial lesions were more acute or showed more tendency toward liquefaction necrosis. Fibrosis in the specimens from patients with unclassified mycobacteria was more marked and more

non-specific in character. Giant cells were somewhat less numerous, but when present seemed to be larger and similar to those seen in Boeck's sarcoid. Fat globules were demonstrated in some of the giant cells. Non-specific inflammation, as manifested by infiltrations of lymphocytes and plasma cells, was more conspicuous in the tissue from patients with unclassified mycobacteria disease than from those with true tuberculosis.

Thus there appeared to be a large area of overlap between the histologic reactions of M. tuberculosis and of the unclassified mycobacteria. They both produce endobronchitis; and probably both produce a tuberculous pneumonia on occasion. However, the unclassified mycobacterial organisms probably produce a lesion somewhat more acute, more exudative, and with more non-specific inflammation and fibrosis.

SUMMARY AND CONCLUSIONS

The clinical, epidemiologic, therapeutic and pathologic data in twenty-five patients with pulmonary lesions, from whom the only known pathogenic organism isolated was an unclassified mycobacterium, suggest that unclassified mycobacterial infection of the lung is a definite clinical entity. In this locality the strains that appeared to have clinical significance were photochromogens and non-chromogens. The clinical and pathological picture is similar in many respects to tuberculosis, but displays a very real difference in the degree of infectiousness and response to treatment.

The origin of infection with the unclassified mycobacteria was not apparent from our studies. We believe that such patients should be isolated from the community and from proved tuberculous patients, at least while the sputum is positive, until more knowledge is obtained regarding man-to-man transmission and sources of infection.

Therapy is not satisfactory with the drugs available at this time, but because of some evidence that pulmonary unclassified mycobacterial disease can be progressive in certain subjects, active infections should be treated.

In addition, we have reviewed the course of fourteen patients with apparently active pulmonary disease, from whom both M. tuberculosis and chromogenic mycobacteria were isolated at various times during their disease. Both the organisms isolated from this group showed a high

level of *in vitro* drug resistance. The clinical course of these patients was indistinguishable from that of far advanced pulmonary tuberculosis, and it was not apparent from our data what role, if any, the unclassified mycobacteria played in their disease.

Acknowledgment: The data on household contacts were very kindly furnished by Dr. Harold Freed, Director, Tuberculosis Control Clinic, Public Health Department, Dallas, Texas. The mycobacterial antigens for skin testing were furnished by the William Buchanan Laboratory of The University of Texas Southwestern Medical School, Dallas, Texas [38]. We wish to express appreciation to Mrs. Joanna Bernard and Miss Maro Speight, of the William Buchanan Laboratory of The University of Texas Southwestern Medical School, for their valuable technical assistance.

REFERENCES

- GRIFFITH, A. S. Atypical tubercle bacilli in human and animal tuberculosis with special reference to those occurring in lupus. *Tubercle*, 5: 569, 1924.
- Beaven, P. W. and Bayne-Jones, S. Mycobacterium, Ryan Strain, isolated from pleural exudate. J. Infect. Dis., 49: 399, 1931.
- Cummins, S. L. and Williams, E. W. An "acid-fast" other than the Koch's bacillus cultivated from sputum. *Tubercle*, 15: 49, 1933.
- Branch, A. A study of acid-fast organisms other than mammalian tubercle bacilli isolated from disease in man. *Tubercle*, 14: 337, 1933.
- PINNER, M. Atypical acid-fast organisms. III. Chromogenic acid-fast bacilli from human beings. Am. Rev. Tuberc., 32: 424, 1935.
- STEENKEN, W., JR. and LANDAU, A. Dissociation of two unusual acid-fast organisms isolated from human sources. J. Infect. Dis., 58: 247, 1936.
- FELDMAN, W. H., DAVIES, R., MOSES, H. E. and ANDBERG, W. An unusual mycobacterium isolated from sputum of a man suffering from pulmonary disease of long duration. Am. Rev. Tuberc., 48: 82, 1943.
- MACCALLUM, P. A new mycobacterial infection in man. I. Clinical aspects. J. Path. & Bact., 60: 93, 1948
- MacCallum, P. A new mycobacterial infection in man. II. Experimental investigations in laboratory animals. J. Path. & Bact., 60: 102, 1948.
- Sissons, H. A. A new mycobacterial infection in man. III. Pathology of the experimental lesions in the rat. J. Path. & Bact., 60: 110, 1948.
- BUCKLE, G. and TOLHURST, J. C. A new mycobacterial infection in man. iv. Cultivation of the new mycobacterium. J. Path. & Bact., 60: 116, 1948.
- 12. Timpe, A. and Runyon, E. H. The relationship of "atypical" acid-fast bacteria to human disease. J. Lab. & Clin. Med., 44: 202, 1954.
- 13. Runyon, E. H. Veteran's Administration, National

- Tuberculosis Association cooperative study of mycobacteria. Am. Rev. Tuberc., 72: 866, 1955.
- mycobacteria. Am. Rev. Tuberc., 72: 866, 1955.
 14. Tarshis, M. S. and Frisch, A. W. Chromogenic acid-fast bacilli from human sources. I. Cultural studies. Am. Rev. Tuberc., 65: 278, 1952.
- MIDDLEBROOK, G., COHN, M. L. and OSTREICHER, R. Chromogenic acid-fast bacilli from human sources. Am. Rev. Tuberc., 72: 693, 1955.
- POLLAK, A. and BUHLER, V. B. The cultural characteristics and animal pathogenicity of atypical acid-fast organisms which cause human disease.
 Am. Rev. Tuberc., 71: 74, 1955.
- PARLETT, R. and YOUMANS, G. P. Antigenic relationships between mycobacteria as determined by agar diffusion precipitin techniques. Am. Rev. Tuberc., 73: 650, 1956.
- GASTAMBIDE-ODIER, M. M., SMITH, D. W., RANDALL, H. M. and KOEVOET, A. O. Comparison of the native lipids of atypical acid-fast bacilli with the lipids of known mycobacterial types. Am. Rev. Tuberc., 75: 843, 1957.
- WAYNE, L. G., KRASNOW, I. and HUPPERT, M. Characterization of atypical mycobacteria and of nocardia species isolated from clinical specimens: Characterization of atypical mycobacteria by means of the microcolonial test. Am. Rev. Tuberc., 76: 451, 1957.
- Bell, J. C. and Riemensnider, D. K. Studies of nontuberculous acid-fast bacilli recovered from human sources. i. Bacteriologic studies. Am. Rev. Tuberc., 76: 683, 1957.
- SHEPPARD, C. C. Behaviour of the "atypical" mycobacteria in Hela cells. Am. Rev. Tuberc., 77: 968, 1958
- 22. Keltz, H., Colton, R. and Lester, W. A characterization of atypical acid-fast bacilli obtained from patients with pulmonary tuberculosis. *Dis. Chest.*, 34: 368, 1958.
- BUHLER, V. B. and POLLAK, A. Human infections with atypical acid-fast organisms. Am. J. Clin. Path., 23: 363, 1953.
- LAGERGRANTZ, R., OLHAGEN, B. and WICKMAN, K. Acid-fast rods in sputum simulating tubercle bacilli. Acta med. scandinav., 147: 50, 1953.
- Gibson, J. B. Infection of the lungs by "saprophytic" mycobacteria in achalasia of the cardia, with report of fatal case showing lipoid pneumonia due to milk. J. Path. & Bact., 65: 239, 1953.
- HALL, W. H. and ERLANDSON, H. Pulmonary granuloma caused by atypical mycobacterium. Transactions of the Thirteenth Conference on Chemotherapy of Tuberculosis, V. A., Army and Navy, 1954, p. 244.
- CHOFNAS, I. and NEWTON, J. Pneumonitis due to chromogenic acid-fast bacilli. Transactions of the Fourteenth Conference on Chemotherapy of Tuberculosis, V. A., Army and Navy, 1955, p. 205
- Young, D. R. Pulmonary disease simulating tuberculosis and caused by chromogenic acid-fast bacilli. *Lancet*, 2: 750, 1955.

- WOOD, L. E., BUHLER, V. B. and POLLAK, A. Human infection with the "yellow" acid-fast bacillus: A report of fifteen additional cases. Am. Rev. Tuberc., 73: 917, 1956.
- FLORENCE, H. Atypical acid-fast (chromogenic) organisms complicating pulmonary disease. Dis. Chest, 30: 250, 1956.
- 31. Wolinsky, E., Smith, M. M., Mitchell, R. S. and Steenken, W., Jr. Atypical chromogenic mycobacteria associated with pulmonary disease. *Am. Rev. Tuberc.*, 75: 180, 1957.
- 32. CROW, H. E., KING, C. T., SMITH, C. E., CORPE, R. F. and STERGUS, I. A limited clinical, pathologic and epidemiologic study of patients with pulmonary lesions associated with atypical acid-fast bacilli in the sputum. Am. Rev. Tuberc., 75: 199, 1957.
- 33. Hensler, N. M., Flanagan, P. and Sprague, E. M. Tuberculosis-like disease caused by chromogenic acid-fast bacilli. *Am. J. Med.*, 26: 376, 1959.
- Lewis, A. G., Jr., Dunbar, F. O., Lasche, E. M., Bond, J. O., Lerner, E. N., Wharton, D. J., Hardy, A. V. and Davies, R. Chronic pulmonary disease due to atypical mycobacterial infections. Am. Rev. Tuberc., 80: 188, 1959.
- 35. Chapman, J. S. and Guy, L. R. Scrofula caused by atypical mycobacteria. *Pediatrics*, 23: 2, 1959.
- WEED, L. A. Recurring migratory chronic osteomyelitis associated with saphrophytic acid-fast bacilli: report of a case of 10 years' duration apparently cured by surgery. Proc. Staff Meet. Mayo Clin., 31: 246, 1956.
- 37. Konno, K., Kurtzman, R., Bird, K. T. and Sharra, A. Differentiation of human tubercle bacilli from atypical acid-fast bacilli. I. Niacin production of human tubercle bacilli. Am. Rev. Tuberc., 77: 669, 1959.
- 38. Chapman, J. S., Guy, L. R. and Speight, M. Simple method for preparation of mycobacterial skin test antigens. To be published.
- 39. V'ENKLE, W. C., LOOMIS, R. N. and JARBEE, J. N. Chromogenic acid-fast bacilli: Their use in vaccinations against tuberculosis in the guinea pig. *Am. Rev. Tuberc.*, 57: 385, 1948.
- 40. Jenkins, D. E., Babar, D., Chofnas, I., Foster, R., Barkley, H. T., Whitcomb, F., Foster, M., Jones, E. and McGee, H. Patterns of disease associated with atypical mycobacteria in Texas. Presented at the National Tuberculosis Association annual meeting, Chicago, May 25, 1959.
- WOLINSKY, E., SMITH, M. M. and STEENKEN, W., JR. Drug susceptibilities of 20 "atypical" as compared with 19 selected strains of mycobacteria. Am. Rev. Tuberc., 76: 497, 1957.
- ROGUL, R., KELLER, R. and CABELLI, V. J. The classification and susceptibilities to chemotherapeutic agents of chromogenic acid-fast bacilli. Am. Rev. Tuberc., 76: 697, 1957.
- STEENKEN, W., JR., SMITH, M. M. and MONTALBINE, V. In vitro and in vivo effect of antimicrobial agents on atypical mycobacteria. Am. Rev. Tuberc., 78: 454, 1958.

An Epidemic of Inhalation Anthrax, the First in the Twentieth Century*

I. Clinical Features

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DURING a ten-week period in 1957, five cases of inhalation anthrax occurred among the employees of an industrial plant in Manchester, New Hampshire, in association with four cases of cutaneous anthrax. Four of the five patients with inhalation anthrax died, one recovered.

During the nineteenth century inhalation anthrax, popularly called "wool-sorters' disease," occurred frequently in workers handling animal hairs, wools or hides, and approximately 200 cases were reported before 1900 [1–3]. In the Bradford district of England, for example, where the animal hair industry was concentrated, Spear [1] reported twenty-three cases of the disease occurring between November 1879 and September 1880. Since 1900, however, only twenty-one sporadic cases and no epidemics have been reported in the world literature [4], a fact which some have attributed to better ventilation of factories [5].

In view of the unusual opportunity to study this disease afforded by the epidemic, an effort was made to collect the clinical data retrospectively. Epidemiologic studies are reported in another paper [6]. The presently described outbreak demonstrates that inhalation anthrax may still be a hazard to workers handling goat hair, and that the disease still is a fulminating one most often terminating fatally.

CASE REPORTS

The greater part of this information was obtained from hospital records and from inter-

views with the physicians and families of the patients. The summaries of the autopsies are derived from a report by Albrink, Brooks, Biron and Kopel [7]; we are most grateful to them for permission to use their data.

Strains of Bacillus anthracis recovered from the patients were examined for macroscopic appearance after eighteen hours' growth on 5 per cent human blood agar plates incubated at 37°c.; for microscopic appearance of a gramstained preparation; for lysis by B. anthracis gamma bacteriophage [8]; and for pathogenicity in white mice. The strains recovered appeared from these tests to be typical of B. anthracis.

Inhalation Anthrax

Patient T. T. (No. 1), † a sixty year old white man, had worked at the mill since 1941, most recently as a noil remover in the combing department. He was in good health, except for an asymptomatic non-toxic nodular goiter present for twenty-five years.

On Tuesday morning, August 27, 1957, backache and headache developed, and the patient left his job at noon. That afternoon the family physician visited him at home and found an oral temperature of 102°F., pulse 100, and blood pressure 110/70 mm. Hg. A few rhonchi were noted on auscultation of the chest. The diagnostic impression was influenza, for which aspirin and codeine tablets were prescribed.

† The numbers in parentheses at the head of each case report correspond to the chronologic order of the onsets of illness, given in Table 1, and serve to identify the cases in the discussion of their epidemiology [6].

^{*} From the New Hampshire State Department of Health and the Communicable Disease Center, Public Health Service, Atlanta, Georgia, U. S. Department of Health, Education, and Welfare. This work was supported by a contract with the U. S. Army Chemical Corps, Fort Detrick, Frederick, Maryland.

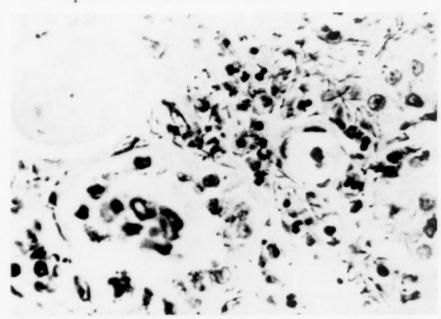


Fig. 1. Patient T. T. Microscopic section (original magnification × 1,000) of the thyroid gland showing many anthrax bacilli in the hemorrhagic interstitial tissue. (Courtesy of Dr. W. Albrink.)

On August 28 the patient's oral temperature was 100°F., and except for a persistent cough he was improved. However, he stayed home from work and on the following day, August 29, his neck suddenly became considerably swollen in the area of the goiter. During the day the swelling did not appear to discomfort the patient, who are normally and was ambulatory. In the evening, however, the patient's condition suddenly worsened. The swelling spread from his neck to his chest. Perspiration was so profuse that five changes of bed clothing were required. The physician was summoned again at 11:00 P.M. and on noting that the patient was afebrile with an irregular pulse and cold, clammy, tremulous hands, administered 5 drops of Lugol's iodine solution. During the next four hours the patient fainted twice. He was admitted to the Elliot Hospital at 4:45 A.M., August 30. During positioning for an emergency x-ray film of the chest such severe respiratory stridor developed that the film could not be made and tracheostomy was considered. After being placed in an oxygen tent, the patient was less dyspneic. His temperature was 102°F., blood pressure 100/60 mm. Hg, apical pulse 92, and respirations 24. On auscultation of the chest moist basilar rales were heard bilaterally. Digitalis and Demerol® were given, and his breathing improved further. However, at 6:00 A.M. he refused a bedpan and walked to the lavatory, where he suddenly became extremely cyanotic, collapsed, and died.

At autopsy the entire mediastinum was occupied by a hemorrhagic edema infiltrating all soft tissues. Scattered throughout this edematous mass were enlarged, hemorrhagic lymph nodes. The parenchyma of the lungs showed a slight increase in consistency which was due to congestion and edema rather than pneumonitis. The trachea was deviated to the right by the swollen lymph nodes and by the left lobe of the thyroid gland, which was enlarged by extensive hemorrhage. Bilateral pleural effusion and splenomegaly were present. The cerebrospinal fluid was clear and colorless.

Gram-positive bacilli were seen in sections of mediastinal lymph nodes, thyroid (Fig. 1) and spleen, and cultures yielded pure growth of B. anthracis.

Patient A. L. (No. 8), a thirty-three year old white man, began working at the mill on August 26, 1957, as a noil remover in the combing department. In his medical history were two hospital admissions: the first for anxiety state with gastric somatization; the second (December 1955) for early hepatic cirrhosis.

The present illness began on October 30, with chills, fever, cough, malaise and generalized muscular aches. He stayed at his job through October 31, but that evening he remained at home and was seen by his physician. Physical examination revealed a temperature of 104°F., slight rhinorrhea, and a non-productive cough. The posterior pharynx was moderately reddened, and occasional wheezes were heard on auscultation of the chest. Profuse sweating was the most striking physical finding.

The physician diagnosed Asian influenza and left the patient 8 to 10 tablets (200,000 units) of oral penicillin. It is uncertain how many tablets were actually taken.

On November 2, the patient appeared improved; his throat was only slightly sore, and he perspired less. That night he slept well, but the following day a sud-

den change took place. Marked diaphoresis recurred, and he had difficulty swallowing saliva. He complained of tightness in the chest, and regurgitation followed attempts to take liquids. The family physician arrived at 1:00 P.M. and found the patient lying motionless in bed, oriented but uncooperative, with his jaws clamped tightly. Dyspnea was not observed, but rhonchi were heard bilaterally over the upper lobes. At 2:00 P.M. the patient became agitated, began to rub his legs, and complained of inability to breathe. Dyspnea increased and cyanosis became evident.

The admitting physician at the Manchester Veterans' Administration Hospital found the patient to be in extremis: delirious, extremely dyspneic, cyanotic and exhibiting marked diaphoresis. On auscultation of the chest many moist rales and wheezes were heard. The possibility of a larvngeal obstruction was considered and a clear airway was seen on direct laryngoscopy; the mucosa was red and injected, but no edema was noted. Morphine, atropine, oxygen, aminophylline, lanatoside C and norepinephrine were administered, and 150 cc. of blood were removed by venesection, all without effect. The patient died at 3:30 P.M.

At autopsy the mediastinum, the mediastinal lymph nodes and the lungs were infiltrated by hemorrhage and edema, and there was bilateral pleural effusion. Several areas of necrosis, hemorrhage and edema were noted in the ileum, but the mesenteric lymph nodes were not enlarged. The brain was covered by a diffuse hemorrhagic leptomeningitis. Microscopically, many gram-positive bacilli were seen in the lungs, meninges, liver, kidney and spleen, and B. anthracis was cultured from the lung, brain and heart's blood. Staphylococcus aureus (phage type 6/47/53/54/77/VA4) and non-hemolytic streptococci were also found in the lung and blood cultures.

Patient A. J. (No. 2), a forty-nine year old white man, had worked at the mill as a card-fixer since June 1956. He had no history of serious illness. In early August 1957 a dry cough developed which he attributed to an increase in the amount of dust in the carding room.

On September 1, 1957, the patient's cough worsened and he became febrile. During the next few days he complained of discomfort in his chest and of anorexia, but continued to work. After attending church on the morning of September 5, he returned home apparently well, but shortly thereafter he was found lying in bed mumbling unintelligibly. His oral temperature was 103°F. A physician was called, who diagnosed a severe cold, possibly bronchitis. An intramuscular injection of 800,000 units of aqueous procaine penicillin and 1 gm. of dihydrostreptomycin was given. At 4:00 P.M. his wife was unable to awaken the patient. At 6:00 P.M. he had a temperature of 104°F. and was incontinent of feces. The patient was unconscious on admission to St. Joseph's Hospital in

Nashua, New Hampshire, with a temperature of 105°F., rapid and shallow respirations, and rales audible over both lungs. A lumbar puncture revealed bloody cerebrospinal fluid; a diagnosis of cerebral hemorrhage was made on that basis. At midnight on September 5, another injection of 800,000 units of penicillin and 1 gm. of dihydrostreptomycin was given. He died at 6:00 A.M. on September 6 without regaining consciousness.

At autopsy a diffuse hemorrhagic leptomeningitis with an exudate containing large numbers of polymorphonuclear leukocytes and gram-positive bacilli, morphologically resembling B. anthracis, was found. No culture was made from the brain, but on staining the bacilli reacted strongly with fluorescent antibody prepared against capsular substance of known B. anthracis organisms [10]. There were small foci of soft consolidation about the hila of both lungs, which microscopically revealed congestion, edema and some hemorrhage into the alveoli and the walls of the bronchioles. The mediastinal lymph nodes were edematous, and there was a left-sided pleural effusion.

Patient E. C. (No. 3), a sixty-five year old white woman, had been employed at the mill since 1946 as a bobbin cleaner in the weaving department. Aside from an asymptomatic non-toxic nodular goiter the

past medical history was negative.

She complained of malaise on September 2, 1957. On the following day she was fatigued but went to work, and on September 4 a mild pain in the chest and a cough developed. She visited the company dispensary on September 5 where an oral temperature of 99°F., a pulse of 92 and a respiratory rate of 24 were noted. The next day she complained of upper abdominal pain for which she consulted her family physician; his impression was possible cholecystitis.

On admission to the Elliot Hospital in Manchester the patient's oral temperature was 95.6°F., pulse 100, respiratory rate 24 and blood pressure 110/90 mm. Hg. The white blood cell count was 16,400 per cu. mm., with a differential of 76 per cent neutrophils, 11 per cent band forms, 9 per cent lymphocytes and

4 per cent monocytes.

On the following morning, while in the radiology department for a cholecystogram, the patient became acutely ill with marked dyspnea and stridor. She was seen by a medical consultant who noted a thready pulse, a frequent, tight, brassy cough without production of sputum, marked stridor (like a child with the croup), and profuse diaphoresis. She was afebrile and had a blood pressure of 90/0 mm. Hg. There was dullness to percussion over the base of the left lung; coarse basilar rales were heard bilaterally. No distention of jugular veins or edema of the ankles was noted. An electrocardiogram showed small QRS complexes and T wave inversion without alteration of the S-T

A portable x-ray film of the chest showed medias-



Fig. 2. Patient E. C. Roentgenogram taken approximately twenty-two hours before death. Mediastinal enlargement, bilateral pleural effusion and perihilar streaking extending to the periphery are seen.

tinal enlargement and perihilar streaking extending to the periphery of the lungs. (Fig. 2.) The base of the lungs were obscured by pleural effusion, and interlobar effusion was present in the fissure of the middle lobe of the right lung. The cholecystogram taken previously did not demonstrate an abnormality.

A diagnosis of cardiac failure with superimposed pneumonia was made. Administration of digitalis, diuretics and bronchodilators was begun at 3:00 p.m. on September 7, but the patient became progressively more dyspneic and perspired profusely; the blood pressure remained at 90/0 mm. Hg. At midnight her pulse was weak and thready, with a rate of 142; her respiratory rate was 32. One hundred milligrams of cortisone and 400,000 units of aqueous procaine penicillin were given intramuscularly and the same dose of penicillin was repeated at 8:00 a.m. on September 8. After twelve hours of marked dyspnea and cyanosis despite continuous oxygen therapy, the patient died at 10:40 a.m. Permission for a postmortem examination was refused.

Patient L. L. (No. 4), a forty-six year old white man, had been employed at the mill since October 1955 as a card tender. His previous medical history was non-contributory.

The patient felt a slight malaise on September 9, 1957. Three days later, September 12, there was a sudden onset of fever (103°F.), chills, cough, dyspnea and profuse diaphoresis. He was seen at home by one of the authors (M. U.), who gave him an injection of

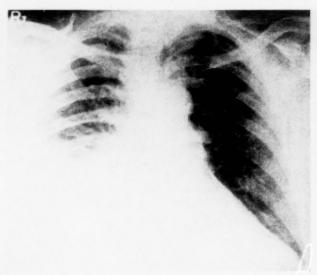


Fig. 3. Patient L. L. Roentgenogram taken on the fourth day of illness. Consolidation of lower lobe of right lung is evident.

400,000 units of aqueous procaine penicillin and recommended immediate hospitalization.

On admission to the Sacred Heart Hospital in Manchester, he was an acutely ill, slightly cyanotic and dyspneic man who coughed frequently and appeared to be confused. His pulse was 108, respirations 32 and blood pressure 120/80 mm. Hg. His conjunctivas were slightly reddened and his pharynx had a single small ulceration in the left tonsillar area. The tongue was heavily coated. There was dullness to percussion over the base of the right lung and moist expiratory rales were heard over the same area. The hemoglobin was normal, the white blood cell count was 9,900 per cu. mm., with 60 per cent neutrophils, 24 per cent band forms and 16 per cent lymphocytes.

Urinalysis was negative except for 8 to 10 white blood cells per high power field of centrifuged sediment. A sputum specimen cultured shortly after admission yielded a non-hemolytic, coagulasenegative Staph. albus. Subsequent blood cultures were negative. A roentgenogram of the chest taken on September 12 showed a patchy consolidation involving the posterior and middle division of the lower lobe of the right lung. (Fig. 3.)

A combination of 800,000 units of procaine penicillin and 1 gm. of dihydrostreptomycin was administered intramuscularly at 4:00 p.m. and was repeated every twelve hours thereafter. During the evening the patient continued to cough and appeared extremely apprehensive. It was necessary to change his bed linen frequently, due to excessive diaphoresis. A second sputum specimen obtained on September 13, subsequent to two inoculations of penicillin and dihydrostreptomycin, yielded a pure culture of non-hemolytic, coagulase-negative Staph, aureus.

The patient's temperature on September 14 was 101°F.; on September 15 he was afebrile, and by

September 19 he felt almost well. However, roentgenographic examination showed no resolution of the consolidation in the right lung. A repeat white blood cell count September 23 was 15,600 per cu. mm. with 69 per cent polymorphonuclear leukocytes, 10 per cent bands, 17 per cent lymphocytes and 4 per cent monocytes.

A roentgenogram taken on October 1 revealed a moderate amount of pleural fluid in the right thorax without resolution of the consolidation. A thoracentesis was performed and 300 ml. of sterile blood-tinged fluid were removed.

A second thoracentesis on October 4 yielded a small amount of frankly bloody fluid. Papanicoloustained preparations of both fluids were negative, and Wright-stained preparations showed numerous red blood cells with occasional lymphocytes and polymorphonuclear leukocytes but no bacteria. From the second pleural fluid "Bacillus subtilis" was recovered. The identification was based on a gram stain of the nutrient broth culture. Since the technician believed the organism a contaminant, the slide and culture were discarded without additional identification.

Another roentgenogram taken on October 5 showed early resolution of the consolidation. Bronchoscopy revealed only diffusely hyperemic bronchial mucosa. A second strength PPD skin test was negative.

Roentgenographic examination on October 25 demonstrated more clearing of the opacity in the lower lobe of the right lung. By January 11, 1958, there was only slight x-ray evidence of residual disease in the lower lobe of the right lung. The patient has remained asymptomatic since his discharge and has returned to work.

Serologic studies were performed on serums obtained twenty-seven and sixty-four days after the onset of illness (earlier specimens were not available). A complement fixation test against a crude filtrate of a culture of B. anthracis showed a titer of 1/30 in the first serum and 1/60 in the second. Control serums have not given titers over 1/15 [9].

A precipitin test using the agar double diffusion technic [10] showed titers of 1/8 in both serums against purified protective antigen from anthrax culture filtrates [11]. Titers of 1/8 have been found also in persons immunized by protective antigen: demonstrable titers have not been found in unimmunized persons and those not exposed to anthrax organisms [12]. By chance, a serum* obtained from this patient in 1953 was available, which gave no titer against anthrax antigen.

Finally, the patient's serum prevented anticapsular fluorescent labeled antibody from reacting with anthrax organisms: the inhibition titer was 1/80 in the first serum and 1/40 in the second. Seven control serums were negative [13].

* Kindly provided by Dr. E. S. Murray, Harvard School of Public Health.

Cutaneous Anthrax

Patient H. T. (No. 6), a thirty-five year old white man, had been employed at the mill since 1950 as a card feeder. On October 10, 1957, the patient noticed a pimple on his forehead, unassociated with pain or pruritus. Oxytetracycline ointment was applied to the lesion by the mill nurse. On the following day a ring of ervthema surrounded the dark center of the lesion; the patient was afebrile but complained of headache. The company physician diagnosed cutaneous anthrax, and prescribed tetracycline (250 mg. every six hours). A culture taken on this day was negative for B. anthracis. On October 14 a black eschar 1 cm. in diameter with slightly raised edges was noted within the lesion. Granulation tissue slowly filled in the anthrax lesion and the patient made an uneventful recovery.

Patient R. P. (No. 7), a fifty year old white woman, had worked as a weaver at the mill since January 1956 and had always been in good health. On October 15, 1957, she noted a pimple on her right wrist which itched and burned but was painless. By October 17 the pimple appeared to contain pus. The patient was seen at the company dispensary October 18, where a diagnosis of cutaneous anthrax was made. Benzathine penicillin (1,200,000 units). oral penicillin and local oxytetracycline ointment were prescribed. A culture of the lesion was positive for B. anthracis. By October 23 the lesion was approximately 1 cm. in diameter, with a tan-red central area surrounded by a slightly elevated, pink ring of vesicular tissue. Subsequently the patient made an uneventful recovery and lost no working days due to the illness.

Patient V. K. (No. 5), a sixty-four year old white woman, had been employed at the mill since 1950 as a weaver and had always been in good health. On October 8, 1957, the patient noticed a small pimple on the first phalanx of the fifth finger of the right hand. The lesion was pruritic but not painful. The pimple gradually became larger and by October 14 the entire finger was swollen. On that day a clinical diagnosis of cutaneous anthrax was made at the company dispensary. A culture of the lesion yielded B. anthracis. Antibiotic therapy was instituted in the form of 1,200,000 units of benzathine penicillin administered intramuscularly and local oxytetracycline ointment. Despite treatment the patient's hand was red and swollen on October 15. Within several days, however, the finger and hand returned to normal size and the eschar slowly disappeared.

Patient C. S. (No. 9), a healthy sixty-one year old man, had worked at the mill since August 16, 1957, most recently as a card tender. On November 5 the patient noted a small pimple on his chest approximately 2 inches below the sternal notch. He did not

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report to the company dispensary until November 11, at which time a 2 by 1 cm. black eschar with surrounding erythema was noted. The culture obtained on this date was negative for B. anthracis. The patient received 1,200,000 units of benzathine penicillin and local oxytetracycline ointment, and made an uneventful recovery.

COMMENTS

Two patients (T. T. and A. L.) had bacteriologically and pathologically proved inhalation anthrax. In the third patient (A. J.), bacteria were found in histologic sections which morphologically and antigenically resembled B. anthracis. The negative postmortem blood culture in this patient may have been the result of antibiotic treatment which had been given before death.

The evidence for the diagnosis of inhalation anthrax in patient E. C. is clinical and epidemiologic. The course of her illness and the roentgenographic appearance of her thorax were consistent with the disease. Epidemiologically, the fact that the fatal illness occurred at the same time and was accompanied by the same symptoms as proved cases of the disease bolsters the diagnosis of inhalation anthrax.

The case of patient L. L. is of unusual interest because of the rarity of recovery from inhalation anthrax [14,15]. His clinical course was similar to those of the proved cases, with one notable difference: apparent localization of disease to one lobe of the lung rather than diffuse involvement. The increase in parenchymal infiltration shown on x-ray examination concomitant with complete amelioration of symptoms is reminiscent of the cutaneous anthrax lesion, which progresses in its typical evolution despite sterilization by antibiotics.

The possible significance of the recovery of "B. subtilis" from the pleural fluid of this patient need hardly be pointed out. Failure to isolate anthrax bacilli from the blood or sputum of the patient would weigh against the diagnosis but for the fact that the cultures were taken after penicillin had been given. The staphylococci which were recovered from the blood and sputum cultures were coagulase-negative. It is possible that these organisms were secondary invaders in an anthrax infection (see patient A. L.).

The time and place of infection constitute epidemiologic evidence for the diagnosis of inhalation anthrax inasmuch as the patient worked in the card-comb room, as did most of the epidemic patients.

Complement fixation, agar precipitin and indirect fluorescent antibody tests all showed the presence of antibodies to B. anthracis in convalescent serums. At least two separate antigens were involved: the anthrax protective antigen in the complement fixation and agar precipitin tests, and the capsular antigen in the fluorescent antibody inhibition test. Recent serologic studies [12] indicate that antibodies detectable by the agar diffusion precipitin inhibition test are found in a significant percentage of persons who are in continual contact with anthrax spores, but who give no history of illness. Although previous inapparent infection may explain the presence of anthrax antibodies in the serum of patient L. L., the occurrence of a pulmonary illness justifies the tentative conclusion that this was a case of inhalation anthrax.

The symptoms, signs and course of the four cases of cutaneous anthrax were typical of the disease [16,17]. All the patients incurred their lesions on exposed body surfaces. Antibiotic treatment, although it sterilized the lesions, did not alter the progression to eschar formation.

Symptoms and Signs of Inhalation Anthrax. A characteristic clinical picture of inhalation anthrax can be derived from the literature [1–5]. The course of disease is typically in two stages, as seen in Figure 4. The initial stage is marked by the insidious onset of a mild fever, malaise, fatigue, myalgia, non-productive cough, and frequently a sensation of precordial oppression. There are few objective findings aside from fever. Rhonchi may be heard on auscultation of the lungs. This initial stage typically lasts for several days. Slight improvement in the clinical condition of the patient may be seen towards the end of the first stage.

The second stage of the disease develops suddenly with acute dyspnea and subsequent cyanosis. The patient appears moribund, with accelerated pulse and respiration. The body temperature, although usually elevated to 102° f. or more, may be subnormal because of shock. Stridor occurs commonly, perhaps as a result of partial extrinsic obstruction of the trachea by enlarged mediastinal nodes. Profuse diaphoresis is a frequent sign, and subcutaneous edema of the chest and neck may be present. On examination of the chest, moist crepitant rales and signs of pleural effusion are observed. Although the spleen is usually enlarged at autopsy, clinical

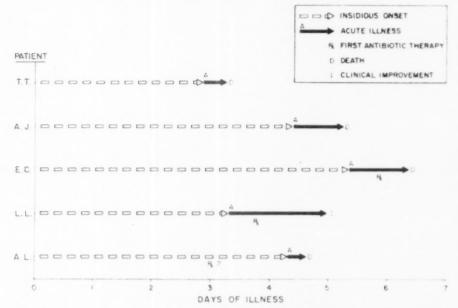


Fig. 4. Diagrammatic representation of the two stages of inhalation anthrax exemplified by the Manchester patients: insidious onset $(--\rightarrow)$ and acute toxemia $(A\rightarrow)$. The occurrence of first antibiotic treatment (Rx), death (D) or improvement (I) are shown in temporal relationship to these stages.

reports rarely mention its palpability. Consciousness typically is maintained until death, but should meningeal involvement occur, there will be disorientation, coma and meningismus. Trismus is a characteristic sign of anthrax meningitis [18].

The average duration of the acute stage is less than twenty-four hours, at the end of which death usually occurs. It is interesting that in-

Table 1
CLINICAL CHARACTERISTICS OF CASES OF INHALATION
ANTHRAX

Z11	Patient				
Characteristic	Т. Т.	A. L.	A. J.	E. C.	L. L
Stage of insidious onset:					
Duration (days)	3	4	4	5	3
Fever	+	++	+	0(5)	+
Malaise	++	++	+	++	++
Precordial oppression	0	+	+	+	0
Stage of acute toxicity:					
Duration (hr.)	10	7	18	25	*
Dyspnea	++	++	+	++	++
Fever Stridor	+++	++	++	++	++
Diaphoresis	+	++	0	+	++
Pleural effusion	++	+	+	+	+
Disorientation	0	++	++	0	+
Trismus	0	+	0	0	0

Note: + = Characteristic present

++ = Characteristic present in severe form.

0 = Characteristic absent.* Patient recovered gradually.

halation anthrax also causes sudden death in such diverse species as the cow [19], the guinea pig [20] and the chimpanzee [21].

The identity of the clinical courses of the Manchester patients with the typical one cited is indicated in Table 1.

Pathogenesis. The pathogenesis of inhalation anthrax in animals has been studied extensively in recent years. Ross [22] has shown that the spores of B. anthracis are ingested as inert particles by macrophages in the alveoli. The spore-bearing macrophages migrate through the alveolar membrane into the lymphatics, and are carried to the hilar lymph nodes. According to Barnes [23], only 0.1 per cent of the spores of a virulent strain reach the nodes of guinea pigs in a viable state. Here germination of the spore may take place, followed by multiplication of the vegetative form. Multiplication of the bacillus is accompanied by hemorrhage and edema of the lymph nodes and surrounding mediastinal connective tissue. Inhalation anthrax is not primarily a pneumonic disease; parenchymatous infiltration of the lungs if present is usually the result of secondary infection by other bacteria. The pleural effusion which occurs is caused perhaps by lymphatic obstruction [3,24].

Eventually, bacilli gain access to the blood stream via the lymphatics [25] with a resulting septicemia and subsequent involvement of the meninges, spleen and intestines. The lepto-

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meninges are frequently diffusely hemorrhagic, and contain neutrophils and anthrax bacilli. The spleen as a rule is enlarged and teeming with organisms. Necrotic ulcerations in the small intestine are sometimes found as a result of bacterial multiplication in the lymphoid tissue of the intestinal wall. The work of Smith, Keppie and associates [20,26,27] has shown that in guinea pigs death from anthrax is caused by the release into the blood plasma of a specific toxin. Once a sufficient level of toxin has been attained, death almost invariably follows, despite sterilization of the animal by antibiotics. In guinea pigs the toxin produces shock due to the loss of blood volume from internal hemorrhage and from edema of the thoracic structures. The bacterial capsule, composed of glutamyl polypeptide, participates in the course of the infection by acting as an antiphagocytic substance [28].

The pathologic findings in the three autopsied Manchester cases are summarized in Table II. No common pathologically demonstrable entities have been discovered which increase the susceptibility of persons to inhalation anthrax. Two of the Manchester patients had nontoxic nodular goiters and one had early hepatic cirrhosis, but no single disease was common to all the patients aside from anthrax.

Diagnosis. The premortem diagnosis of inhalation anthrax is difficult. The first stage is often mistaken for influenza or bronchitis, and the second stage resembles cardiac failure or cerebrovascular accident. Viewed as a whole, however, the clinical course is characteristic of the disease. The diagnosis should be strongly considered in a person who has had recent contact with infected materials, and who suddenly becomes severely dyspneic or comatose following several days of mild febrile illness. All goat hair, wool or hides imported from the Middle or Far East should be presumed to contain the anthrax bacillus, a presumption which is at least 50 per cent correct [29]. Laboratory workers in contact with the organism also are at risk.

B. anthracis has been cultured from the blood, cerebrospinal fluid and sputum of patients with inhalation anthrax before death [14,18,30,31]. In view of the characteristic appearance of the anthrax bacillus, gram-stained smears of blood, sputum and cerebrospinal fluid should be prepared [17,30,32]. Demonstration of mediastinal widening by roentgenography is highly sugges-

TABLE II
PATHOLOGIC FINDINGS IN AUTOPSY CASES

	Patient			
Pathologic Characteristic	т. т.	A. L.	A. J.	
Edema of chest wall	++	+	0	
Hemorrhagic mediastinitis	++	++	0	
Mediastinal lymphadenitis	++	++	+	
Pleural effusion	++	+	+	
Intestinal lesions		+	0	
Mesenteric lymphadenitis	0	0	0	
Splenomegaly		0	+	
Hemorrhagic meningitis	0	++	++	
B. anthracis				
cultured from tissues	Yes	Yes	No	
Gram-positive bacilli				
seen in tissues	Yes	Yes	Yes	

NOTE:

0 = Characteristic not present.

+ = Characteristic present.

++ = Characteristic present in severe form.

tive of inhalation anthrax, in the presence of other signs and a history of exposure.

It should be noted, however, that several cases have occurred in which no contact with materials containing anthrax spores was discovered [33,34]. These patients are thought to have been infected by stray spores from nearby plants handling animal hairs or hides. Infection by contagion has not been reported in human inhalation anthrax [1].

Prognosis and Treatment. During the nineteenth century in the textile mills of England, 20 per cent of patients with inhalation anthrax were said to recover spontaneously [1]. Only two of the approximately nineteen recovered cases which have been reported in detail, however, had cultural evidence for the diagnosis [14,15]. Asymptomatic B. anthracis bacteremia has been observed in chimpanzees [21], and in the light of serologic evidence suggesting that inapparent or mild forms of anthrax occur in man [12], spontaneous recovery may not be as unlikely as the dearth of case reports would suggest. The recovery of patient L. L. thus may have been unrelated to treatment, particularly in view of the fact that two of the other patients (A. J. and E. C.) also received parenteral antibiotics. On the other hand, the development of meningitis in one, and the delay in beginning antibacterial treatment of the other patient may account for the failure of antibiotics in these cases.

In any event, the use of antibiotics seems clearly indicated in other suspected cases of inhalation anthrax. In workers exposed to anthrax spores, lower respiratory or influenzal illnesses should be suspected of being the first stage of anthrax infection. Although appropriate cultures should be taken first, antibiotic treatment should be given without waiting for the results. This precaution seems justified by the severity of the disease, and by experiments in which eradication of B. anthracis infections in guinea pigs did not prevent a fatal outcome once the stage of toxemia and severe symptoms had developed [27].

On the basis of limited numbers of animal experiments, penicillin can be expected to cure inhalation anthrax if given before the onset of toxemia [23,35]. It is of interest to note that Keppie et al. [20] reported inability to stop anthrax bacteremia in guinea pigs by means of tetracyclines or chloramphenicol, in contrast to success with streptomycin (penicillin was too toxic for the guinea pig). Although the anthrax organism is sensitive in vitro to all common antibiotics except polymyxin B [17,36], until more complete in vivo studies are reported, penicillin or penicillin and streptomycin are the drugs of choice because of their bactericidal properties. Only moderate doses of antibiotics presumably are necessary, since the organism is sensitive in vitro to 0.03 units per ml. of penicillin and 1.25 μg . per ml. of streptomycin [36].

Anthrax hyperimmune serum made in horses was used with apparent success before the advent of antibiotics in the treatment of systemic anthrax secondary to cutaneous lesions [37]. In view of the importance of toxemia to the outcome of the experimental disease, the use of antitoxin seems logically indicated in human inhalation anthrax. Unfortunately, antiserum is no longer available commercially in the United States.

During the severe stage of the disease, measures for the support of any patient in shock should be undertaken.

SUMMARY

Five cases of inhalation anthrax and four cases of cutaneous anthrax are presented. Three inhalation and two cutaneous cases were proved by culture or microscopic demonstration of the anthrax bacillus, and the others were clinically consistent with the diagnosis. These cases constituted an epidemic, for they all oc-

curred within a ten-week period at a goat hair processing mill.

The illnesses of the patients with inhalation anthrax began insidiously with mild fever, fatigue and malaise lasting several days. This mild initial phase was terminated by the sudden development of dyspnea, cyanosis and, in two cases, disorientation and coma. Four patients died within twenty-five hours of the onset of severe symptoms.

The principal lesions found by pathologic examination of three patients were hemorrhagic mediastinitis and lymphadenitis, pleural effusion, acute splenitis and, in two cases, hemorrhagic meningitis.

The pathogenesis, symptoms, diagnosis and treatment of inhalation anthrax are discussed.

Physicians should consider this disease when faced with an acute febrile illness in a person occupationally exposed to anthrax spores.

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REFERENCES

- Spear, W. Tenth Annual Report of the Local Government Board. London, 1880.
- Bell, J. H. On woolsorters' disease. Lancet, 1: 871, 1880.
- EPPINGER, H. The Ragpickers' Disease, A Typical Respiratory Infection of Man. Jena, 1894. Gustav Fischer.
- SPENDLOVE, J. C., BRACHMAN, P. S., ALBKINK, W. and PLOTKIN, S. A. Inhalation anthrax: a review. Manuscript in preparation.
- Bell, J. H. and Legge, T. M. In: The System of Medicine, vol. 2, pp. 227–257. Edited by Albutt and Rolleston. London, 1906. Macmillan Co.
- BRACHMAN, P. S., PLOTKIN, S. A., BUMFORD, F. H. and Atchison, M. A. An epidemic of inhalation anthrax. п. Epidemiologic investigation. Am. J. Hyg., 72: 6, 1960.
- 7. Albrink, W., Brooks, S., Biron, R. and Kopel, M. Human inhalation anthrax: a report of three cases. Am. J. Path., 36: 457, 1960.
- BROWN, E. R. and CHERRY, W. B. Specific identification of Bacillus anthracis by means of a variant bacteriophage. J. Infect. Dis., 96: 34, 1955.
- 9. McGann, V. U. S. Army Chemical Corps Labora-

AMERICAN JOURNAL OF MEDICINE

- tories, Fort Detrick, Frederick, Md. Personal communication.
- THORNE, C. B. and Belton, F. C. An agar diffusion method for titrating Bacillus anthracis immunizing antigen and its application to a study of antigen production. J. Gen. Microbiol., 17: 505, 1957.
- THORNE, C. B. U. S. Army Chemical Corps Laboratories, Fort Detrick, Frederick, Md. Personal communication
- NORMAN, P. S., RAY, J. G., BRACHMAN, P. S., PLOTKIN, S. A. and PAGANO, J. S. Serologic testing for anthrax antibodies in workers in a goat hair processing mill. Am. J. Hyg., 72: 32, 1960.
- processing mill. Am. J. Hyg., 72: 32, 1960.
 13. CHERRY, W. B. and FREEMAN, E. M. Staining bacterial smears with fluorescent antibody. v. The rapid identification of Bacillus anthracis in culture and in human and murine tissues. Zentralb. Bakt., 175: 582, 1959.
- LODGE, S., JR. La maladie des trieurs de laine. Arch. méd. expér. et anat. Path., 117: 241, 1890.
- KRONBERGER, H. Ein Fall von Lungenmilzbrand mit günstigen Ausgang. Klin. Tubercular Berl., 38: 135, 1917
- Ellingson, H. V., Kadull, P. J., Bookwalter, H. L. and Howe, C. Cutaneous anthrax. Report of twenty-five cases. J. A. M. A., 131: 1105, 1946.
- Gold, H. Anthrax—a report of 117 cases. Arch. Int. Med., 96: 387, 1955.
- POLAND, J. Internal anthrax. Tr. Path. Soc. London, 37: 550, 1886.
- 19. ALBRINK, W. Personal communication.
- KEPPIE, J., SMITH, H. and HARRIS-SMITH, P. W. The chemical basis of the virulence of Bacillus anthracis. III. The role of the terminal bacteremia in death of guinea pigs from anthrax. *Brit. J. Exper. Path.*, 36: 315, 1955.
- ALBRINK, W. S. and GOODLOW, R. J. Experimental inhalation anthrax in the chimpanzee. Am. J. Path., 35: 1055, 1959.
- 22. Ross, J. M. The pathogenesis of anthrax following the administration of spores by the respiratory route. J. Path. & Bact., 73: 485, 1957.
- Barnes, J. M. The development of anthrax following the administration of spores by inhalation. *Brit.* J. Exper. Path., 28: 385, 1947.

- Greenfield, W. S. Eleventh Annual Report of the Local Government Board, pp. 207–226. London, 1881
- WIDDICOMBE, J. G., HUGHES, R. and MAY, A. J. The role of the lymphatic system in the pathogenesis of anthrax. *Brit. J. Exper. Path.*, 37: 343, 1956.
- SMITH, H., KEPPIE, J., STANLEY, J. L. and HARRIS-SMITH, P. The chemical basis of the virulence of bacillus anthracis. IV. Secondary shock as the major factor of death of guinea pigs from anthrax. Brit. J. Exper. Path., 36: 323, 1955.
- SMITH, H., KEPPIE, J. and STANLEY, J. L. The chemical basis of the virulence of Bacillus anthracis. v.
 The specific toxin produced by B. anthracis in vivo.
 Brit. J. Exper. Path., 36: 460, 1955.
- 28. SMITH, H., ZWARTOUW, H. T. and HARRIS-SMITH, P. W. The chemical basis of the virulence of Bacillus anthracis. VIII. Fractionation of the intercellular material of Bacillus anthracis. Brit. J. Exper. Path., 37: 361, 1956.
- 29. Brachman, P. S. and Fekety, F. R. Industrial anthrax. Ann. New York Acad. Sc., 70: 574, 1958.
- REECE, R. J. Anthrax: simulating cerebro-spinal fever. Lancet, 1: 406, 1910.
- 31. BROOKSHER, W. R. and BRIGGS, J. A. Pulmonary anthrax: report of a case. J. A. M. A., 74: 323, 1920.
- 32. Schottmüller, F. Ueber Lungenmilzbrand. München. med. Wchnschr., 45: 1231, 1898.
- Levinsky, W., Anderson, P. and Richardson, G. Inhalation anthrax, meningitis, and bacillemia. Symposium on Anthrax in Man, pp. 96–103. Philadelphia, 1954. Pennsylvania State Department of Health.
- 34. Brachman, P. S., Pagano, J. S. and Albrink, W. Inhalation anthrax: report of two cases, one in a patient with sarcoidosis. To be published.
- 35. Henderson, D. W., Peacock, S. and Belton, F. C. Observations on the prophylaxis of experimental pulmonary anthrax in the monkey. *J. Hyg.*, 54: 28, 1956.
- GARROD, L. P. The sensitivity of Bacillus anthracis to antibiotics. Antibiotics & Chemother., 2: 689, 1952.
- Lucchesi, P. Serum treatment of nineteen cases of anthrax, including one of external, internal, and bacteremic type. Am. J. M. Sc., 183: 795, 1932.

New Observations on the Sympathetic Postganglionic Mechanism*

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It will be remembered that W. B. Cannon spent several years determining what substance was released into the blood when the postganglionic sympathetic fibres were stimulated. He finally came to the conclusion that there were two substances, one which he called sympathin E and the other sympathin I. Since the work of Euler [16] there has been general acceptance of the view that norepinephrine is the principal substance released. Hitherto everyone has assumed that this release is similar to the release of acetylcholine by the nerves of the parasympathetic system, and that the extent of the release is determined only by the impulses passing down the nerves.

Norepinephrine in the Tissues. In tissues innervated by postganglionic sympathetic fibres norepinephrine is present and can be extracted from them. This was first shown in blood vessels [27]. It was then demonstrated for the heart [19] and for the spleen, the liver, the kidney and the salivary glands [18]. The extractable norepinephrine disappeared when the sympathetic fibres degenerated [18]. Euler [17] considered that the norepinephrine must be present in the terminations of the sympathetic fibres, but if so the amount would be very large. He calculated that it would be in the range 3 to 30 mg. per gm., whereas the amount in the splenic nerves before they reach the spleen is about 15 μ g. per gm., that is to say one thousand times less.

During the time since these observations were made no importance has been attached to the presence of norepinephrine in these organs, and it has not been connected in any way with the action of the sympathetic nerves. Recently, however, it was shown [3] that when the alkaloid reserpine was injected into a rabbit, the injection was followed after sixteen hours by a disappearance of norepinephrine from the heart. Similar

observations were made for other organs [11,14]. Thus when rabbits and dogs were treated with reserpine on two successive days, and then killed on the third day, the extractable norepinephrine disappeared from the aorta. Similarly in cats the extractable norepinephrine disappeared from the spleen and from the iris of the eye. The changes produced are shown in Table 1.

Effect of Treatment with Reservine. The effect of removing the extractable norepinephrine was first observed in the heart by dissecting the atria and setting them up in an isolated organ bath. Atria from rabbits treated with reserpine contracted at a slower rate than atria from normal rabbits although the conditions were the same. Since this furnished the main direct evidence that the extractable norepinephrine exerted an effect in the absence of nerve stimulation, the observations are given in Table II. The mean rate of normal atria was 146 per minute and that of atria from animals treated with reserpine was 112 per minute. This difference was highly significant (P less than 0.001), and it indicated that the norepinephrine present in the heart exerted an effect upon the pacemaker; it was not inactive [10].

Observations were also made on the effect of stimulating the accelerator fibres to the heart. Huković [20] prepared the atria of the rabbit heart with the sympathetic fibres attached, suspending the atria in a bath. He stimulated the postganglionic fibres as they left the stellate ganglion. In a preparation from a normal rabbit, stimulation increased the rate. In a preparation from a rabbit treated with reserpine he found that stimulation had either no effect or an inhibitory effect. The inhibitory effect was abolished by atropine. The main finding was that the sympathetic fibres lost their accelerator action when the extractable norepinephrine dis-

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Table 1
Change in Mean amount (µg./gm.) of extractable Norepinephrine in organs after treating animal with reserpine (total dose 5 mg./kg.)

Organ	Normal	After Reser- pine	Authors
Heart (rabbit)	1.57	0.03	Bertler, Carlsson and Rosengren
Aorta (rabbit)	0.47	0.11	Burn and Rand
Aorta (dog)	0.95	0.03	Burn and Rand
Spleen (cat)	1.13	0.12	Burn and Rand
Iris (cat)	0.17	0.01	Burn and Rand

appeared as a result of treating the rabbit with reserpine.

Observations were also made on the effect of stimulating the sympathetic fibres to the vessels of the dog's hind leg. The hind leg was perfused with blood by using a Dale-Schuster pump which started the perfusion at the moment of stopping the natural circulation. When the preparation was made from a normal dog, stimulation of the lumbar sympathetic chain of the corresponding side caused constriction of the vessels of the leg, and a consequent rise of pressure in the cannula through which blood entered the vessels; there was also a diminution in the venous outflow. When the preparation was made from a dog treated with reserpine, stimulation of the sympathetic chain caused only vasodilatation in the vessels of the leg; this was abolished by atropine, and in the presence of atropine stimulation had no effect.

Restoration by Norepinephrine. These observations on the heart and blood vessels made it clear that the cardiovascular action of reserpine was peripheral. The early findings led to the view that reserpine caused a fall of blood pressure as a result of its central action [2], but no central effect could have been present in the isolated atria or the perfused dog's hind leg. Finally it has been shown that reserpine does not cause any depression of the central sympathetic outflow [21].

Although postganglionic sympathetic stimulation did not accelerate the heart or constrict the blood vessels in preparations from an animal treated with reserpine, sympathetic stimulation recovered its normal action after the intravenous infusion of norepinephrine. An increase of sympathetic action as a result of an intravenous

Table II

SPONTANEOUS RATE OF RABBIT ATRIA IN ISOLATED ORGAN. BATH AT 30°C. (BEATS PER MIN.)

Control Atria	Atria from Rabbits Treated with Reserpine
132	128
132	136
164	116
168	112
170	100
144	92
136	104
152	108
168	138
	100
	104
Mean: 146	112

infusion of epinephrine was previously observed by Burn [7]. This increase, however, was slight. When, however, norepinephrine was added to the blood perfusing the hind leg of a dog treated with reserpine, and allowed to act during 30 to 40 minutes, the vessels constricted. About 0.5 mg. of norepinephrine was added to 1 L. of blood. When all the norepinephrine had been added the vasoconstriction gradually diminished and the vessels returned to their previous state. Stimulation of the sympathetic postganglionic fibres at this point produced constriction. Thus the infusion of norepinephrine restored the normal effect of sympathetic stimulation [12].

Similar observations were made in the cat when the contractions of the nictitating membrane were recorded. This has a sympathetic innervation, and stimulation of the post-ganglionic fibres leaving the superior cervical ganglion normally causes contraction. However, when the cat was previously treated with reserpine, stimulation had little effect. If, however, an intravenous infusion of norepinephrine was given during 30 minutes, then when the effect of this norepinephrine on the blood pressure had disappeared, sympathetic stimulation once more caused a large contraction of the nictitating membrane.

Action of Precursors of Norepinephrine. Not only norepinephrine but also its precursors, dopamine and L-dopa restored the effect of sympathetic stimulation on the iris of the eye and on the nictitating membrane in the cat treated with reserpine. Dopamine, or 3-hydroxytyramine,

requires the introduction of an OH group on the carbon atom of the sidechain next to the ring in order to become norepinephrine. L-Dopa is the amino acid from which dopamine is formed by decarboxylation. The total amounts infused were 10 mg. of dopamine, and 30 mg. of L-dopa.

Action of Norepinephrine in the Normal Animal. Finally it was found that the effect of sympathetic stimulation on the vessels of the dog's hind leg was increased by an infusion of norepinephrine into the normal animal, without previous treatment with reserpine.

These observations were made both in the dog anaesthetised with chloralose and in the perfused hind leg. The anaesthetised dog was eviscerated and a device was used to keep its blood pressure constant. This was a bottle containing heparinised blood from another dog, connected to the left external iliac artery. Since the blood in the bottle was under air pressure equal to the dog's blood pressure, vasoconstriction in the dog served only to force blood into the bottle, and vasodilatation only withdrew blood from the bottle. The blood pressure did not change.

The right sympathetic chain was prepared for stimulation, and vasoconstriction in the right hind leg was measured by a plethysmograph fitted to the leg. The plethysmograph was sufficiently sensitive to record the changes in leg volume which occurred with each heart beat. The strength of stimulus (in milliamps) just sufficient to cause vasoconstriction was then determined, atropine being injected to exclude vasodilator effects. When this had been done, an infusion of norepinephrine was given during 30 minutes, and then during the following 60 minutes the threshold stimulation for vasoconstriction was redetermined. It was found to have fallen appreciably; in a series of experiments the threshold fell to a mean value of 40 per cent of the initial threshold. Thus in one experiment the minimum strength for vasoconstriction was 1.05 ma. at the beginning; after an infusion of norepinephrine this fell to 0.15 ma. Similar results were obtained in perfusing the dog's hind leg when no anaesthetic was present [15].

The Question of a Norepinephrine Store. Evidence was thus obtained that norepinephrine, slowly infused into the blood stream, in some way increased the effect of a given stimulus applied to the postganglionic sympathetic fibres. At the point of stimulation the sympathetic chain was tied and there was no circulation through vessels running between the fibres. The norep-

inephrine could not therefore have increased the sensitivity of the sympathetic fibres at the point of stimulation. Once an impulse in a fibre is initiated it obeys the all or none law, and therefore we may conclude that the impulses arriving at the ends of the fibres in response to a given stimulus were the same after the infusion of norepinephrine as before. The observations therefore suggest that at the postganglionic termination there is a store of norepinephrine which can be added to by norepinephrine circulating in the blood, and that the effect of a given stimulus applied to the sympathetic chain depends on the size of the store. If this suggestion is correct, it would indicate a function for the norepinephrine which is secreted by the adrenal medulla into the blood. There has long been doubt of the purpose of this secretion since it was first demonstrated [5]. If, however, the norepinephrine secreted into the blood stream can be withdrawn from the blood and stored in the neighborhood of the sympathetic nerve endings it may provide part of the norepinephrine which is discharged by impulses passing down the postganglionic fibres.

Clinical Significance of the Store. The evidence that there is a store from which sympathetic impulses release norepinephrine may be of importance in relation to hypertension. For example, it is known that in those with excessive secretion of norepinephrine from a phaeochromocytoma, who begin with intermittent crises of hypertension, a permanently raised blood pressure may develop. This may happen because the amounts of norepinephrine released during the crises fill up the store, so that sympathetic impulses then have a greater effect than in a normal subject. While knowledge is at present too scanty to make speculation worth while, it can at least be pointed out that the fall of blood pressure which follows treatment with reserpine is almost certainly due to diminution of the store.

An observation of immediate practical importance concerns the effect of the store on sensitivity to norepinephrine. In the course of some surgical operations when the blood pressure falls to an undesirably low level, it is fairly common practice to give an intravenous drip containing norepinephrine. The blood pressure can then be maintained at a higher level. However, difficulties sometimes arise when the intravenous drip is stopped, because the blood pressure may fall very low, and when this happens the drip is usually restarted. Cases have been described in

which the drip has been continued for several days. We have demonstrated that when a drip of norepinephrine is given in this way, while the effect of postganglionic stimulation increases, the blood vessels become more and more insensitive to the action of norepinephrine in the blood. The amount of constriction produced becomes less and less. However, there are other pressor amines available, such as ephedrine, metaraminol and mephentermine, and the effect of these is either not much less than normal or it is actually increased as the result of the norepinephrine drip. It appears that amines like ephedrine act by releasing norepinephrine from the store, and are most efficient when the store is full. When it is found that the blood pressure falls dangerously after giving a norepinephrine drip, the correct treatment is not to continue the drip but to give an injection, either intravenous or intramuscular, of a pressor amine like ephedrine. In animals given a continuous infusion of norepinephrine these amines have a prolonged effect [13].

Agents Causing Thrombosis. There is evidence that agents causing vascular thrombosis may do so as a result of an action which involves the store of norepinephrine; it begins with vasoconstriction due to the liberation of norepinephrine from the store. Thus there are cases on record of the injection of thiopental having been made by accident into an artery. This has resulted in arterial spasm, and in ischaemia of the part supplied; later the vessels have been found to be thrombosed. Thiopental was studied in the vessels of the perfused rabbit ear, in which it was shown to cause vasoconstriction. This vasoconstriction, like that due to norepinephrine, was increased in the presence of cocaine, and blocked in the presence of tolazoline (Priscoline®). The vasoconstriction was reduced or absent in the ears of rabbits previously injected with reserpine [8].

Other substances, such as quinine sulphate, ethanolamine and sodium iodide, which are known to have caused vascular thrombosis clinically, were observed to have a similar action to that of thiopental. Substances which are used for the purpose of obliterating varicose veins may act in this way, for norepinephrine can be extracted from veins as well as from arteries [27].

Peripheral Action of Nicotine. An interesting outcome of these investigations is the explanation of certain peripheral actions of nicotine exerted at points beyond the site of sympathetic

ganglia, actions which are yet sympathomimetic in character. Thus if the atria of the rabbit heart are dissected and set up in an isolated organ bath so that they can contract, the addition of nicotine to the bath has two effects. First there is inhibition due to stimulation of the parasympathetic ganglia in the atria. This is excluded in the presence of atropine, and then acceleration of the rate and force of the contractions is seen. This acceleration is similar to that produced by adding norepinephrine to the bath. It does not occur if the rabbit has been injected with reserpine beforehand. We learn therefore that nicotine can liberate norepinephrine from atrial tissue, and we may conclude that certain effects which smoking has in promoting cardiac arrhythmias in susceptible patients may be due to this effect.

Effects of nicotine have been recorded on the peripheral vessels. Thus if the vessels of the isolated rabbit ear are perfused with a modified Ringer's solution, the injection of a small amount of nicotine into the vessels causes vasoconstriction. This vasoconstriction is no longer seen if the ear is removed from a rabbit which has been treated with reserpine, so that the vasoconstriction is due to the release of norepinephrine. This evidence was supported by the demonstration that norepinephrine was present in the skin of the normal rabbit ear, but was absent in the skin of the ear when the rabbit was treated with reserpine [11].

The significance of these findings lies in the observations that the smoking of one or two cigarettes can cause a very striking fall in skin temperature when the subject is in warm surroundings [23]. The findings also appear to be related to the disease thromboangiitis obliterans, which becomes acute when people smoke. There are many physicians who think it essential that patients with Buerger's disease should not smoke.

The Location of the Store. If Euler is right in thinking that the norepinephrine which can be extracted from the spleen is present in the terminal branches of the postganglionic fibres, the store would then appear to be within these fibres. There is however at least one other possibility. Adams-Ray and Nordenstam [1] have described the presence of chromaffin cells in human skin, and their findings have been confirmed [6]. Chromaffin cells resemble the cells of the adrenal medulla in their staining characteristics. Their occurrence in the body

elsewhere than in the adrenal medulla has been known for many years, and their distribution was described by Kohn (1903) who found them present throughout the sympathetic system [22]. Pines [26] described them as being innervated. Muscholl and Vogt [24] found them in the inferior mesenteric and solar ganglia of the cat but not in the stellate ganglia. These workers recorded the presence of very large amounts of norepinephrine and epinephrine in the inferior mesenteric and solar ganglia, and attributed this to the presence of the chromaffin tissue on the surface of the ganglia. In the cat they found that treatment with reserpine depleted the norepinephrine content of the sympathetic neurones quite readily, but did not so easily deplete the chromaffin tissue.

When the observations on the rabbit ear were made, the skin of the ear was examined histologically using the methods described by Nordenstam and Adams-Ray [25]. Cells corresponding to their description were found, and they were absent from the skin of rabbits treated with reserpine. Similar cells were found in the skin of the cat's tail and in the nictitating membrane of the cat's eye. Again these cells were absent in the animal treated with reserpine, and they were absent in the nictitating membrane after degeneration of the nerves [9].

These observations clearly open up a wider field for chromaffin cells than that described by Kohn. Kohn's cells have been regarded as containing epinephrine, but it may be that those present in the skin contain norepinephrine. What relation they may have to the postganglionic sympathetic fibre is quite undetermined.

Recently Boura et al. [4] have introduced a new type of blocking agent, Darenthin, which can diminish or abolish the effect of stimulating postganglionic sympathetic fibres, although it does not diminish the action of norepinephrine or epinephrine. In particular it does not diminish the action of tyramine which releases norepinephrine from the store. The action of Darenthin could be explained as due to the block of a synaptic connexion between the postganglionic fibre and the store. If the store were in chromaffin cells the innervation would in some respects resemble that of the cells of the adrenal medulla by the fibres of the splanchnic nerve.

SUMMARY

We have known for about ten years that norepinephrine can be extracted from the heart and from blood vessels; hitherto this norepinephrine has been regarded as an inert store. When animals are treated with reserpine, this extractable norepinephrine disappears, and stimulation of postganglionic fibres no longer causes acceleration of the heart or constriction of the blood vessels.

However, if norepinephrine is given by slow intravenous infusion the normal effect of post-ganglionic stimulation returns. Slow intravenous infusion of the precursors of norepinephrine, namely dopamine and L-dopa, has the same restoring effect.

If an infusion of norepinephrine is given to a normal animal the effect of postganglionic stimulation is increased, and the threshold falls. The facts are consistent with the view that impulses passing along the postganglionic fibres liberate norepinephrine from a store, and that the effect of a given stimulation depends on the size of the store. The norepinephrine secreted by the adrenal medulla may serve to maintain the store and, if the secretion is too great, may contribute to hypertension.

Continuous infusion of norepinephrine, as by intravenous drip, causes desensitization of the vessels to norepinephrine present in the blood. This explains the difficulty of maintaining the blood pressure by intravenous drip of norepinephrine. However, vessels which respond less and less to norepinephrine respond well to the injection of amines like ephedrine or metaraminol or mephentermine, which act by releasing norepinephrine from the store.

Nicotine releases norepinephrine from the heart and from the blood vessels. This explains the fall in skin temperature on smoking, and the reason for patients with Buerger's disease not to smoke.

Various substances which have been observed to cause vascular thrombosis when injected into arteries or veins have the power to release norepinephrine from vessel walls.

REFERENCES

- ADAMS-RAY, J. and NORDENSTAM, H. Un system de cellules chromaffines dans la peau humaine. Lyon chir., 52: 125, 1956.
- Bein, H. J. Significance of selected central mechanisms for the analysis of the action of reserpine. *Ann. New York Acad. Sc.*, 61: 4, 1955.
- Bertler, A., Carlsson, A. and Rosengren, E. Release by reserpine of catecholamines from rabbits' hearts. *Naturwissenschaften*, 43: 521, 1956.
- Boura, A. A., Green, A. F., McCoubrey, A., Laurence, D. R., Moulton, R. and Rosenheim,

AMERICAN JOURNAL OF MEDICINE

- M. L. Darenthin: hypotensive agent of new type. Lancet, 2: 17, 1959.
- BÜLBRING, E. and BURN, J. H. Liberation of noradrenaline from the suprarenal gland. Brit. J. Pharmacol., 4: 202, 1949.
- Burch, G. E. and Phillips, J. H. Chromaffin reacting cells in human digital skin. Circulation Res., 6: 416, 1958.
- Burn, J. H. On vasodilator fibres in the sympathetic and on the effect of circulating adrenaline in augmenting the vascular response to sympathetic stimulation. J. Physiol., 75: 144, 1932.
- BURN, J. H. and HOBBS, R. Mechanism of arterial spasm following intra-arterial injection of thiopentone. *Lancet*, 1: 1112, 1959.
- BURN, J. H., LEAGH, E. H., RAND, M. J. and THOMPson, J. W. Peripheral effects of nicotine and acetylcholine resembling those of sympathetic stimulation. J. Physiol., 148: 332, 1959.
- Burn, J. H. and Rand, M. J. Action of nicotine on the heart. *Brit. M. J.*, 1: 137, 1958.
- BURN, J. H. and RAND, M. J. Noradrenaline in artery walls and its dispersal by reserpine. *Brit. M. J.*, 1: 903, 1958.
- Burn, J. H. and Rand, M. J. The action of sympathomimetic amines in animals treated with reserpine. J. Physiol., 144: 314, 1958.
- Burn, J. H. and Rand, M. J. Fall of blood pressure after a noradrenaline infusion and its treatment by pressor agents. *Brit. M. J.*, 1: 394, 1959.
- BURN, J. H. and RAND, M. J. The cause of the supersensitivity of smooth muscle to noradrenaline after sympathetic denervation. J. Physiol., 147: 135, 1959.
- Burn, J. H. and Rand, M. J. Sympathetic post-ganglionic mechanism. Nature, London, 184: 163, 1959

- v. EULER, U. S. The presence of a substance with sympathin E properties in spleen extracts. Acta physiol. scandinav., 11: 168, 1946.
- v. Euler, U. S. Noradrenaline. Springfield, Ill., 1956. Charles C Thomas.
- v. EULER, U. S. and PURKHOLD, H. Effect of sympathetic denervation on the noradrenaline and adrenaline content of the spleen, kidney and salivary glands in the sheep. Acta physiol. scandinav., 24: 212, 1951.
- GOODALL, McC. Studies of adrenaline and noradrenaline in mammalian heart and suprarenals. Acta physiol. scandinav., supp. 85, vol. 24, 1951.
- 20. Huković, S. Isolated rabbit atria with sympathetic nerve supply. *Brit. J. Pharmacol.*, 14: 372, 1959.
- IGGO, A. and VOGT, M. The effect of reservine on the electrical activity in preganglionic sympathetic fibres. J. Physiol., 147: 14 P., 1959.
- 22. Kohn, A. Die Paraganglien. Arch. mikr. Anat., 62: 263, 1903.
- 23. McDevitt, E. and Wright, I. S. The Biologic Effects of Tobacco. Edited by Wynder, E. L. Boston, 1955. Little, Brown & Co.
- Muscholl, E. and Vogt, M. The action of reserpine on the peripheral sympathetic system. *J. Physiol.*, 141: 132, 1958.
- 25. NORDENSTAM, H. and ADAMS-RAY, J. Chromaffin granules and their location in human skin. Ztschr. f. Zellforsch., 45: 435, 1957.
- PINES, I-L. J. Über die Innervation des chromaffinen Gewebes des Sympathicus und über das sympathico-chromaffine System in allgemeinen. Arch. Psychiat., 70: 636, 1923.
- Schmiterlow, C. G. The nature and occurrence of pressor and depressor substances in extracts from blood vessels. *Acta physiol. scandinav.* (supp. 56), 16: 1, 1948.

Nitrogen Mustard and the Steroid Hormones in the Treatment of Inoperable Bronchogenic Carcinoma*

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Among the malignant neoplasms for which a chemotherapeutic or systemic form of treatment is urgently needed is bronchogenic carcinoma. Its incidence is steadily rising [1]. Four thousand new cases were admitted to Veterans Administration Hospitals in 1958 [2]. The vast majority of the patients are first seen only after the tumor has become unresectable, and the cure rate even of the small number of patients found operable is small [3].

The large number of these patients in the Veterans Administration Hospitals presents both a challenge and an opportunity to attempt a systematic evaluation of drugs currently available so that a firm basis of comparison might be established for use in the study of the newer cancerocidal drugs. This could best be accomplished by a cooperative study involving several of the larger hospitals to insure a sufficient number of patients so that statistically reliable conclusions can be reached relatively quickly.

Since it was estimated that more than half the patients admitted to Veterans Administration Hospitals with inoperable bronchogenic carcinoma die within three months [4], prolongation of life should be satisfactory as a measure of drug effectiveness, thus avoiding difficulties inherent in subjective evaluation. Five drugs were selected for this first study†—nitrogen mustard, diethylstilbestrol, testosterone propionate, progesterone and cortisone—to be compared with a regimen

in which the patient received the same intensive supportive care but without any additional specific agent. Except for nitrogen mustard and testosterone propionate, the drugs were given orally as coded agents by a double-blind technic. The patients on the non-specific regimen received lactose.

While nitrogen mustard has long been thought to be effective in treating the syndrome of superior vena caval occlusion arising during the course of lung cancer, and also the lytic bone lesions produced by the tumor, its ability to prolong life has never been adequately studied in a well controlled series [5,6]. Such data appeared to us to be essential before any of the newer drugs could be studied profitably.

Since lung cancer occurs at least four to five times more frequently in males than in females it had been suggested by some earlier investigators that diethylstilbestrol or perhaps even orchiectomy might halt the progression of disease [7]. In very small groups of patients these treatments had no effect. However, testosterone propionate used to test the hypothesis, instead of making the patients worse, seemed to relieve bone pain in some [8,9]. One investigator reported exceptionally good results in a series of five patients treated with testosterone combined with cortisone [10]. These drugs therefore also were included in the present study. Progesterone was added to the protocol since it too had been thought to be of beneficial effect in the treatment of carcinomas ordinarily responsive to the sex hormones (cervix, breast and prostate) [11],

[†] Drugs supplied through the courtesy of The Upjohn Company, Merck Sharp & Dohme, and Syntex Chemical Company, Inc.

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TABLE 1
TOTAL NUMBER OF PATIENTS ADMITTED TO STUDY

Drug Used	No Previous Therapy	With Resection	With Radiation	Removed	Total	
Nitrogen mustard	53	10	7	10	80	
Diethylstilbestrol	64	12	6	7	89	
Inert compound	62	16	6	4	88	
Testosterone propionate	74	18	7	7	106	
Cortisone	55	17	6	8	86	
Progesterone	67	10	6	8	91	
Total	375	83	38	44	540	

and would complete our study of sex hormone

Cortisone has appealed to many investigators interested in the treatment of bronchogenic carcinoma, not only for its non-specific effects but also because experiments had indicated that the growth of many animal tumors could be inhibited by the use of the adrenal corticosteroids [12]. However, in animals there is a divergence of effects since cortisone appears to facilitate heterologous transplantation of tumors, and even when the primary tumor is inhibited metastases develop in much greater numbers than in the controls [13-31]. Clinically, the corticosteroids do appear to have some effect on leukemias and lymphomas [32-35], but little or no response has been recorded in bronchogenic carcinoma except for the suggestion of subjective improvement in a few patients [36-42]. Since even this is well worth achieving until a better form of therapy becomes available, cortisone was added to our trial series in an attempt to confirm the clinical suggestion.

The principal investigators from the fourteen Veterans Administration Hospitals agreed to participate under the chairmanship of Dr. James W. Hollingsworth and accepted patients into the study between February 17, 1958 and February 16, 1959. All patients with primary carcinoma of the lung who could not benefit from surgery or radiation therapy were included if there was either biopsy proof of the diagnosis of carcinoma of the lung or satisfactory cytologic evidence. All morphologic types of carcinoma were included.

Patients who had received either radiation therapy or antineoplastic drugs were accepted provided there was no residual toxicity from the prior treatment and the patient had either not responded or had relapsed after some temporary improvement of any sort. Patients who had had "curative" surgery but had evidence of recurrence or in whom only palliative resection had been performed were also included.

A complete history and physical examination were obtained in each patient in addition to indicated x-ray examinations and other laboratory studies. When it was determined that a patient met the criteria for inclusion in the study, treatment was begun. During the period of treatment the patients were examined twice weekly and sufficient laboratory studies were carried out to guard against development of corticosteroid toxicity as well as to follow the course of illness.

In addition to the specific therapeutic regimen, the patient was given the best possible supportive treatment. Antibiotics, transfusions and other measures were given when necessary. Palliative radiation to extrathoracic sites for relief of pain or radiation to the superior mediastinum because of superior vena caval obstruction was given to a small number of patients without dropping them from the study. However, if it became necessary to deliver radiotherapy to the primary lesion, subsequent observations on the patient were not included in the analysis.

Nitrogen mustard was given intravenously in a single dose of 0.4 mg./kg. body weight and repeated six weeks later if there was no evidence of hematologic toxicity. Testosterone propionate was given intramuscularly in 100 mg. dosages three times a week for a period of twelve weeks. The three other drugs and the inert compound (lactose) were identical in physical form and were given orally, two tablets four times a day after meals for twelve weeks and then reduced by one tablet weekly thereafter. This gradual reduction in dosage was effected since one of the drugs was cortisone and it was felt desirable to decrease its dosage gradually to prevent acute adrenal insufficiency. Cortisone was thus given in a dose of 100 mg. a

TABLE II
SOURCE OF TISSUE FROM WHICH POSITIVE HISTOLOGIC DIAGNOSIS WAS MADE

Diagnosis	Bronchus	Lymph Node	Lung via Thoracotomy	Öther Sites	Total
Squamous carcinoma	127	26	31	32	216
Undifferentiated small cell carcinoma	57	27	17	12	113
Adenocarcinoma	9	22	17	9	57
Undifferentiated large cell carcinoma	29	26	13	14	82
Total	222	101	78	57	468

day; progesterone, 2 mg. a day; and diethylstilbestrol, 10 mg. daily. Code names were assigned to the oral drugs. The code was held by the Clinical Pharmacology and Therapeutics Section of the National Cancer Institute.

Randomization of treatments was carried out for each hospital separately. All patients were divided into three basic groups: (1) those who had received radiation therapy previously, (2) those who had had surgical resection, and (3) those who were termed "without previous therapy" (including a few patients who had received antineoplastic drugs). Within each group, treatments were assigned by unrestricted randomization. After the randomization program was completed each investigator was supplied with three series of sealed envelopes, numbered consecutively and designating treatments. Pooled results of randomization are given in Table 1 which shows the number of patients under each treatment regimen.

Of the forty-four patients removed from the study, in eight instances treatment was not started because of the death of the patient. Fourteen inadvertently

Table III · Classification of types of bronchogenic carcinoma

	No. of Cases	Total
Squamous carcinoma		216
Keratinizing	28	
Moderately well differentiated	110	
Poorly differentiated	78	
Undifferentiated small cell carcinoma.		113
Oat-cell type	81	
Polygonal-cell type	32	
Adenocarcinoma		57
Glandular	40	
Papillary	7	
Poorly differentiated	10	
Undifferentiated large cell carcinoma		82
Total		468

received other forms of treatment concurrently. The diagnosis was not confirmed in eleven cases, and in another eleven the primary site was found to be other than the lung.

Three pathologists independently examined the original slides upon which the diagnosis was made. Since the pathologist in the originating hospital had first examined the slides, in most cases four opinions were available. Each pathologist classified the material as squamous cell carcinoma, adenocarcinoma or undifferentiated carcinoma, which was subdivided into large cell and small cell and the latter further subdivided into oat-cell and polygonalcell types. Smears of sputums, bronchial secretions or pleural fluid were read as positive or negative for carcinoma cells. When doubtful, they were classified as negative. The final diagnosis rested upon the agreement of at least two of the three pathologists. When there was no agreement as to type, the tumor was classified as undifferentiated large cell carcinoma.

The diagnosis was obtained from a bronchial biopsy specimen in 47 per cent of the patients (Table II), squamous carcinoma being the most common tumor in this group. The diagnosis of adenocarcinoma was made most frequently by lymph node biopsy. In Table III there is an analysis of the types of bronchogenic carcinoma found in this series. In fifty-two additional patients the diagnosis was made by cytologic means only and in twenty others the original slides were lost in transit but the patients were included in the analysis of results because of other incontrovertible evidence of bronchogenic carcinoma.

RESULTS

Analysis of the patients' survival times after the start of therapy was made as of August 1, 1959. On this date 420 patients were deceased, sixty-four were still living, and twelve were termed "limited observations." Among those who were alive, the shortest observation time was 171 days; the longest, 514 days. The twelve "limited observations" came about through the patient's receiving added therapy or being lost to

TABLE IV
PROPORTION SURVIVING TO INDICATED DAY

Day	Nitrogen Mustard	Diethyl- stilbestrol	Inert Compound	Testosterone Propionate	Cortisone	Progesterone
-			Point Estimo	ate		
30th	0.87	0.80	0.75	0.75	0.74	0.73
60th	0.75	0.58	0.62	0.62	0.46	0.51
90th	0.63	0.44	0.51	0.44	0.37	0.41
120th	0.51	0.33	0.46	0.37	0.26	0.33
150th	0.42	0.26	0.38	0.28	0.21	0.27
180th	0.39	0.25	0.30	0.22	0.18	0.23
- '		90 F	Per Cent Confidence I	nterval Estimate		
30th	0.78-0.93	0.72-0.87	0.66-0.82	0.66-0.82	0.65-0.82	0.64-0.81
60th	0.65-0.83	0.48-0.67	0.52-0.71	0.53-0.70	0.37-0.56	0.41-0.60
90th	0.53-0.73	0.35-0.54	0.42-0.61	0.36-0.53	0.28-0.47	0.32-0.51
120th	0.39-0.61	0.24-0.42	0.37-0.56	0.29-0.46	0.18-0.35	0.25-0.43
150th	0.32-0.52	0.19-0.36	0.29-0.48	0.20-0.36	0.14-0.30	0.19-0.36
180th	0.29-0.50	0.18-0.34	0.22-0.39	0.16-0.30	0.11-0.27	0.16-0.32

follow-up study. In either case, observations prior to the disqualifying event were used in the analysis.

The five drugs and the inert compound have been compared by considering the proportion of patients who survived to the 30th, 60th, 90th, 120th, 150th and 180th day after treatment was begun. All estimates were made by what Kaplan and Meier have termed the "Product Limit" method [43].

The experience of patients who had been treated previously either by surgery or radiation was analyzed and found to be no different from that of persons without previous therapy. Therefore, the presentation of data has been limited to that for the entire group.

The distribution of the four principal tumor types did not differ statistically among the various drug groups so that the end result could not have been influenced by any possible difference

TABLE V
PROBABILITY OF SURVIVING TO A GIVEN DAY

	Nitrogen Mustard	Diethyl- stilbestrol	Inert Com- pound	Testosterone Propionate	Cortisone	Progesterone
Probability (proportion) of surviving to 30 days: Point estimate		0.80 0.72-0.87	0.75 0.66-0.82	0.75 0.66–0.82	0.74 0.65-0.82	0.73 0.64–0.81
Probability of surviving to 60th day having survived to 30th day Point estimate		0.72 0.61-0.81	0.83 0.71-0.90	0.82 0.73-0.89	0.62 0.50-0.73	0.69 0.58-0.79
Probability of surviving to 90th day having survived to 60th day: Point estimate		0.76 0.63-0.86	0.83 0.71-0.90	0.72 0.61-0.81	0.81 0.66-0.90	0.80 0.67-0.90

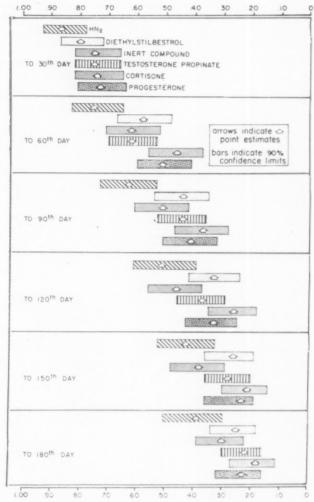


Fig. 1. Ninety per cent confidence interval estimates of the proportion surviving to indicated day.

in responsiveness. No attempt was made to analyze the survival rates of patients with different tumor types.

The proportion of patients who survived to a given time is presented in two ways: (1) the point estimate, and (2) the 90 per cent confidence interval estimate. (Table IV, Fig. 1.) In most instances the point estimate was simply the proportion of patients surviving to the indicated time since, with the exception of the twelve limited observations, all persons were observed for at least 171 days or until death. The confidence interval estimates were obtained by computing the 90 per cent confidence limits for each point estimate. (The probability that the "true" proportion lies somewhere within the range of the interval is 90 per cent.)

With prolongation of life as the measurement, the most favorable experience was among those patients who received nitrogen mustard; the least favorable, among those who were treated with cortisone. For example, only 37 per cent of the patients treated with cortisone survived to the ninetieth day compared to 51 per cent for those who received the inert compound and 63 per cent for those whose therapy was nitrogen mustard. The survival rates at the ninetieth day for diethylstilbestrol, testosterone propionate and progesterone fell between those for cortisone and the inert compound.

The slightly better experience with nitrogen mustard resulted primarily from the fact that a larger proportion of the patients survived the first month. Eighty-seven per cent of the patients receiving nitrogen mustard survived at least thirty days as against 75 per cent of the control patients. (Under the assumption that nitrogen mustard prolonged life to no greater degree than the inert compound, a difference of twelve percentage points would be expected slightly less than 10 per cent of the time.) Once the patients who received nitrogen mustard entered the second month after treatment, their survival was similar to that of the patients who received the inert compound. (Table v. Fig. 2.) The second course of mustard therapy six weeks after the first had no effect on the rate of survival. The transient beneficial effect of nitrogen mustard could be expected from reports in the literature [5,6], but its duration proved to be very short. Even more important is the apparent lack of effect of the second course of the alkylating agent. Apparently nitrogen mustard can exert some cancerostatic influence but the cells may then develop resistance to further dosages.

The possible deleterious effects of cortisone were noted in the second month. At the end of the twenty-ninth day the survival rate among the group receiving cortisone equalled that for the control group. Among the patients who lived to the second month, however, the survival rate during the next thirty days was 62 per cent for those on cortisone as against 83 per cent for those in the control group. The probability that this difference was the result of sampling variation is less than 2 per cent.

In view of the previous reports of the beneficial effects of cortisone, it was surprising to find this increased mortality among those patients receiving it during their second month of treatment. A careful analysis of the major contributory causes of death (Table vi) failed to disclose any increased incidence of heart failure or pulmonary infection which we first thought might be the

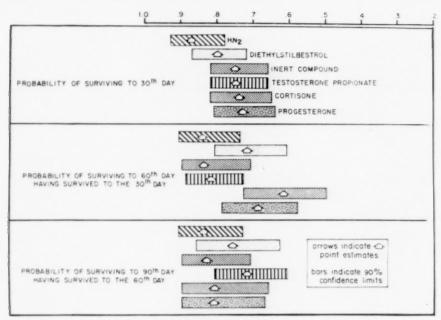


Fig. 2. Conditional probabilities of survival.

explanation. We are unable to account for this increased mortality among the patients treated with cortisone. While one patient did die of perforated ulcer, there was one such death in the group given the inert compound.

It is noteworthy that little to none of the usually observed adrenal corticosteroid side effects were found in our patients receiving 100 mg. of cortisone daily for three months. Actually, no appreciable number of side effects was observed with any of the drugs. During the study gynecomastia developed in a few patients. Some of these, when the code was broken, were found to have received the inert compound rather than the expected diethylstilbestrol. There was some nausea and even vomiting among the patients who received nitrogen mustard but

no serious hematologic complications were encountered.

Also of considerable interest are the thirteen patients who died of pulmonary hemorrhage. Six had received diethylstilbestrol. Five had keratinizing squamous carcinoma (defined as well differentiated) and three had moderately well differentiated squamous carcinoma. In this small series two-thirds of the patients suffering severe hemorrhage had squamous carcinoma, but, more strikingly, five of thirteen had keratinizing squamous carcinoma (in contrast to the general figure of 6 per cent of all biopsies). Since the distribution of the twenty-eight cases of this highly differentiated form of squamous carcinoma was approximately the same throughout the six treatment groups, the use of di-

TABLE VI PRINCIPAL CONTRIBUTORY CAUSES OF DEATH

Drug Used	Total	None	Pulmonary Infection	Hemorrhage	Heart Failure	Perforated Ulcer	Other
Nitrogen mustard	54	36	14	2	2	0	0
Diethylstilbestrol	65	38	16	6	2	0	3
Inert compound	72	47	14	1	4	1	5
Testosterone propionate	87	66	17	0	2	1	1
Cortisone	72	49	15	1	2	1	4
Progesterone	70	46	16	3	2	0	3
Total	420	282	92	13	14	3	16

TABLE VII
MEDIAN SURVIVAL TIMES AND ESTIMATED AVERAGE SURVIVAL TIMES

Survival	Nitrogen Mustard	Diethyl- stilbestrol	Inert Com- pound	Testosterone Propionate	Cortisone	Progesterone
Median survival (days)		75	93	78	56	60
Estimated mean survival (days)		138	138	117	106	122

ethylstilbestrol in keratinizing squamous carcinoma may have led to more rapid necrosis with subsequent hemorrhage.

Except for this possible influence of hemorrhage in patients with keratinizing squamous carcinoma, the sex hormones had no effect on the life span of the patients that could be distinguished from the inert compound. There is the suggestion that progesterone may have exerted almost as deleterious an effect as cortisone during the second month of treatment but the difference from the inert compound was such that its significance was 7 per cent as compared to the 2 per cent significance level for the difference between cortisone and the inert compound.

Observed median survival and the estimated survival times are presented in Table VII. These data add evidence to the only slightly beneficial effect of nitrogen mustard (thirty days longer median survival time than for the inert compound); to the apparently unfavorable results among patients who received cortisone (fifty-six and 106 days as compared to ninety-three and 138 days for the group receiving the inert compound); and to the suggestion that the three sex hormones are of no value in prolonging life.

While the therapeutic implications that can be drawn from this first study by our group are all on the negative side, we have demonstrated that bronchogenic carcinoma can be well studied by the large scale cooperative technic using the proportion of patients surviving to a given day as the index of drug comparison. The advantages of such an end point are obvious when compared to the other commonly used ones of subjective evaluation and/or tumor measurements [44]. Both present many difficulties involving agreement among different investigators and the elimination of many patients whose tumors are not measurable. Long range survival studies are always difficult be-

cause of the large number of patients who have to be dropped because they receive additional treatment or are lost to follow-up study.

SUMMARY

Four hundred ninety-six patients with bronchogenic carcinoma were divided into comparable groups and each treated for three months with nitrogen mustard, diethylstilbestrol, testosterone propionate, cortisone, progesterone or an inert compound (lactose).

Sixty-three per cent of the patients given nitrogen mustard were alive at the end of ninety days as compared to 51 per cent of the patients receiving the inert compound and 37 per cent of the patients treated with cortisone. The first course of nitrogen mustard improved the survival rate at the thirtieth day only. The second course of the drug failed to exert any further effect on survival.

Mortality in the group treated with cortisone was highest during the second month of treatment. No explanation could be found for the apparent deleterious effects of cortisone.

The median survival time for the control group was ninety-three days as compared to 121 days for the group receiving nitrogen mustard and fifty-six days for the patients given cortisone. The estimated mean survival time also confirmed the very slight advantage of nitrogen mustard as compared to the inert compound as well as demonstrating the accelerated death rate among the patients given cortisone.

The results with the other drugs were intermediate between those with the inert compound and cortisone, the experience in the group receiving progesterone being almost as bad as that in the group given cortisone.

Thirteen patients died of exsanguinating hemorrhage; six had received diethylstilbestrol and five had keratinizing squamous carcinoma as compared to an over-all incidence of 6 per

cent of this type of bronchogenic carcinoma in this series.

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REFERENCES

- Budinger, J. M. Untreated bronchogenic carcinoma. A clinico-pathological study of 250 autopsied cases. Cancer, 11: 106, 1958.
- Estimates prepared by the Reports and Statistics Service, Department of Medicine and Surgery, Veterans Administration.
- Lindskog, G. E. and Bloomer, W. E. Bronchogenic Carcinoma. A comparison of two consecutive series of one hundred cases each. *Cancer*, 1: 234, 1948.
- Griswold, Mathew H. et al. Cancer in Connecticut 1935–1951. Connecticut State Department of Health, Hartford, Conn., 1955.
- Roswit, B. Present status of chemotherapy of bronchial cancer. Radiology, 69: 499, 1957.
- KARNOFSKY, D. A., ABELMANN, W. H., CRAVER, L. F. and BURCHENAL, J. H. The use of the nitrogen mustards in the palliative treatment of carcinoma. With particular reference to bronchogenic carcinoma. Cancer, 1: 634, 1948.
- Kemler, R. L. and Graham, E. A. Studies on the influence of sex hormones on successful heterologous transplantation of human bronchogenic carcinoma. *Cancer*, 3: 735, 1950.
- TRUHAUT, R. La chimiothérapie dans le cancer broncho-pulmonaire. Semaine hôp. Paris, 33: 3668, 1957.
- Olson, K. B. A study of certain sex factors and hormone treatment in bronchogenic carcinoma. Am. J. M. Sc., 230: 157, 1955.

 Ornstein, G., Lercher, L. and Robitzek, E. Palliative treatment of lung malignancy with cortisone and corticotropin. Quart. Bull. Sea View Hosp., 12: 125, 1951.

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- TAYLOR, S. G., III, AYER, J. P. and MORRIS, R. S., JR. Cortical steroids in treatment of cancer; observations on effects of pituitary adrenocorticotropic hormone (ACTH) and cortisone in far advanced cases. J. A. M. A., 144: 1058, 1950.
- NOBLE, R. L. Hormonal regulation of tumor growth. Pharm. Rev., 9: 367, 1957.
- Pharm. Rev., 9: 367, 1957.
 13. STOCK, C. C. Anti-tumor activities of steroids in animals. Ciba Foundation Colloquia on Endocrinology, vol. 1, pp. 135–148. London, 1952. J. & A. Churchill.
- 14. Stock, C. C. Experimental cancer chemotherapy. Rec. Progr. Hormone Res., 11: 425, 1954.
- WATSON, B. E. Effects of cortisone and adrenalectomy on the growth rate of the Ehrlich ascites tumor in mice. J. Nat. Cancer Inst., 20: 219, 1958.
- HERBUT, P. A. and KRAEMER, W. H. Heterologous transplantation of human tumors. Cancer Res., 16: 408, 1956.
- 17. Hoch-Ligett, C. and Hsü, Y. T. Heterotransplantation of human tumors into cortisone-treated rats. *Science*, 117: 360, 1953.
- PATTERSON, W. B., CHUTE, R. N. and SOMMERS, S. C. Transplantation of human tumors into cortisonetreated hamsters. Cancer Res., 14: 656, 1954.
- TOOLAN, H. W. Growth of human tumors in cortisone-treated laboratory animals: the possibility of obtaining permanently transplantable human tumors. Cancer Res., 13: 389, 1953.
- TOOLAN, H. W. Transplantable human neoplasms maintained in cortisone-treated laboratory animals: H.S. #1; H.Ep. #1; H.Ep. #2; H.Ep. #3; and H.Emb.Rh. #1. Cancer Res., 14: 660, 1954.
- 21. Bollag, W. and Meyer, C. Heterologous transplantation of tumors. *Oncologia*, 7: 66, 1954.
- 22. Green, H. N. and Whiteley, H. J. Cortisone and tumour growth. Brit. M. J., 2: 538, 1952.
- 23. Foley, E. J. and Silverstein, R. Progressive growth of C3H mouse lymphosarcoma in CF1 mice treated with cortisone acetate (18902). *Proc. Soc. Exper. Biol. & Med.*, 77: 713, 1951.
- 24. Howes, E. L. Cortisone and homologous transplantation of tumors. *Yale J. Biol. & Med.*, 23: 454, 1951.
- 25. Gноse, T. The effect of cortisone on tumor growth and metastasis. *Indian J. M. Sc.*, 12: 629, 1958.
- IVERSEN, H. The influence of cortisone on the frequency of tumour metastases. Acta path. et microbiol. scandinav., 41: 273, 1957.
- LAPIS, K. and SAGI, T. The action of cortisone on the growth and metastasis of transplantable tumors. Orv. Hetel., 97: 268, 1956.
- SKIPPER, H. E. and THOMSON, J. R. VII. Effects of a series of tumor-inhibiting agents and related compounds on L1210 leukemia and drug-resistant line thereof. *Cancer Res.*, 3: 44, 1955.
- 29. Sugiura, K. Studies in a spectrum of mouse and rat tumors. *Cancer Res.*, 3: 19, 1955.
- 30. Clarke, D. A. п. Mouse sarcoma 180. Cancer Res., 3: 14, 1955.
- 31. GELLHORN, A., KELLS, A. and GOLINO, M. VI.

- Mammary adenocarcinoma 755, glioma 26, and Brown-Pearce carcinoma. Cancer Res. (suppl.), 3: 38, 1955.
- 32. Fessas, P., Wintrobe, M. M., Thompson, R. B. and CARTWRIGHT, G. E. Treatment of acute leukemia with cortisone and corticotropin. Arch. Int. Med., 94: 384, 1954.
- 33. Straus, B., Jacobson, A. S., Berson, S. A., Bern-STEIN, T. C., FADEM, R. S. and YALOW, R. S. The effect of cortisone in Hodgkin's disease. Am. J. Med., 12: 170, 1952.
- 34. Lewis, S. M. Cortisone and its analogues in haematology. Postgrad. M. J., 34: 340, 1958.
- 35. Louis, J., Sanford, H. N. and Limarzi, L. R. Treatment of acute leukemia in children and adults. J. A. M. A., 167: 1913, 1958.
- 36. Spies, T. D., Stone, R. E., Lopez, G. G., Milanes, F., Toca, R. L. and Reboredo, A. Response to adrenocorticotropic hormone and cortisone in persons with carcinoma, leukemia and lymphosarcoma. Lancet, 2: 241, 1950.
- 37. GRIBOFF, S. I. The rationale and clinical use of steroid hormones in cancer. Arch. Int. Med., 89: 812, 1952.
- 38. RAAB, A. P. and GERBER, A. Observations on the use of cortisone and ACTH in the management of

- terminal malignancy. New York J. Med., 53: 1333,
- 39. TAYLOR, S. T. The action of ACTH and cortisone in children with disseminated cancer. Proc. Second Clin. ACTH Conf., 2: 230, 1951.
- 40. ALPERT, L. K., ZIMMERMAN, H. S. and SCHERR, E. H. The effects of ACTH, cortisone and nitrogen mustard on malignant diseases, Proc. Second Clin. ACTH Conf., 2: 235, 1951.
- 41. PEARSON, O. H. and ELIEL, L. P. Use of pituitary adrenocorticotropic hormone (ACTH) and cortisone in lymphomas and leukemias. J. A. M. A., 144: 1349, 1950.
- 42. KARNOFSKY, D. A., MEYERS, W. P. L. and PHILLIPS, R. Treatment of the inoperable pulmonary cancer, primary and metastatic. Am. J. Surg., 89: 526, 1955.
- 43. KAPLAN, E. L. and MEIER, P. Nonparametric estimation from incomplete observations. J. Am. Stat. A., 53: 282, 1958.
- 44. Veterans Administration Cancer Chemotherapy Study Group. A clinical study of the comparative effect of nitrogen mustard and DON in patients with bronchogenic carcinoma, Hodgkin's disease, lymphosarcoma, and melanoma. J. Nat. Cancer Inst., 22: 433, 1959.

A Study of the Inverse Relationship Between pKa and Rate of Renal Excretion of Phenylbutazone Analogs in Man and Dog*

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THE analogs of phenylbutazone (4-butyl-1,2-The analogs of phenylodianological constitute diphenyl-3,5-pyrazolidinedione) constitute a group of acidic compounds closely related structurally but differing over a wide range of pKa (acidity) by virtue of appropriate substitutions in the benzene rings and/or butyl side chain of the molecule. In the course of studies on the structure-activity relationships of these analogs it was noted that the potency of the uricosuric properties which they possess was inversely proportional to their pKa [1]. It was further noted that the more acidic compounds (of lower pKa) were excreted rapidly in the urine in man whereas the less acidic compounds (of higher pKa) were very slowly eliminated [2]. This latter finding stimulated a more detailed study of the phenylbutazone series to delineate the mechanisms relating the rate of renal excretion of these acidic drugs to their pKa, and to their uricosuric effects. The results form the subject of this communication.

METHODS

The general experimental design was as follows: Representative phenylbutazone analogs of greater acidity (pKa 2.0 to 3.1) and lesser acidity (pKa 4.5 to 5.5) were selected for study in parallel. The renal excretion of these two series of compounds of varying pKa was first compared in man under the normal circumstances of acid urine, initially in twenty-four-hour urine samples, then more precisely by renal clearance technics. Renal clearance studies of the drugs were then carried out in human subjects before and after alkalinization of the urine by administration

of sodium bicarbonate. The results were subjected to further analysis by appropriate free clearance and stop-flow experiments in the dog.

For the studies of twenty-four-hour urinary excretion of the drugs employed, and their rate of disappearance from the plasma in man, a single intravenous injection, usually of 800 mg., was made. For G-13838 and G-15140 an intravenous dose of 400 mg. was employed, for p-nitrophenylbutazone the dose was 600 mg. G-26924 and G-32170 were given orally in 800 mg. dosage. Blood and urine samples were procured at appropriate intervals.

Renal clearance studies were made in eighteen human subjects by the standard procedures previously employed [3]. In sixteen of these the clearance of more acidic analogs (G-28315, G-32567, G-32642) was measured; in two, less acidic compounds (phenylbutazone and G-13838) were studied. Six experiments with G-28315 were carried out, three at a constant infusion rate to provide constant plasma drug levels, three at increasing infusion rates to establish rising plasma drug levels. In addition, three experiments were performed to determine the clearance of G-28315 before and after alkalinization of the urine with sodium bicarbonate. Similar experiments were made with G-32567 and G-32642. The renal excretion of phenylbutazone and of G-13838, and the effect thereon of alkalinization of the urine with sodium bicarbonate, was studied in three human

Ten experiments were carried out in mongrel dogs, four using standard renal clearance procedures and six using stop-flow technics [4–6], to localize, when possible, the tubular sites of secretion and reabsorption of the drugs. In the standard renal clearance

subjects. The dosages of drugs and the detailed

sequences of infusion are indicated in the tables.

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studies, the renal excretion of G-28315, G-32567, phenylbutazone and G-13838 in acid and/or alkaline urine was studied. A priming intravenous dose varying from 300 to 1,000 mg. was given; sustaining infusions were then maintained at a rate usually of 1 mg./minute. In the two dogs receiving G-28315 in stop-flow experiments, an injection of 300 mg. of the drug was first given and the infusion then sustained at a rate of 1.5 mg./minute; in one instance the urine was acid, in the other alkalinized by a prior injection of sodium bicarbonate. Two stop-flow experiments with phenylbutazone, with priming doses of 300 mg. and 600 mg., respectively, followed by a sustaining solution delivering the drug at a rate of 1 mg./minute, were carried out in alkalinized urine. A similar study was made with G-13838 and G-32567, using 600 mg. and 300 mg., respectively, to prime and a sustaining dose of 1 and 2 mg./minute, respectively.

Sulfinpyrazone, phenylbutazone and oxyphenbutazone were determined in plasma and urine as previously described [7-9]. In brief, acidified plasma or urine was first extracted with ethylene dichloride, heptane or a mixture of heptane with 1.5 per cent isoamyl alcohol. For determinations in urine the organic phase was then shaken with an equal volume of pH 4.8 buffer (0.1 M citrate, 0.2 M phosphate) to remove the major portion of the urine "blank," with negligible loss of the drug. The drug was then extracted from the organic phase into 2.5 N NaOH and measured spectrophotometrically. G-13838 (265 mu*) and G-26924 (262 mu) were determined by the method previously employed for phenylbutazone; G-15140 (267 mu) and G-32170 (262 mu) by a method previously described for G-25671 [10]; G-27463 (293 mu) by the procedure used for oxyphenbutazone. G-29701 (266 mμ) and G-32642 (253 mμ) were determined like sulfinpyrazone except that a pH 4 buffer wash was used. For the determination of G-32567 (295 m_µ) and G-31442 (264 m_µ) the sulfinpyrazone method was employed except that 0.5 ml of 6 N HCl was added to the plasma and the mixture was extracted with the organic solvent for two hours. The methods for determination of the analogs in plasma and urine gave 90 per cent or better recoveries.

The specificity of the methods for the urinary assay of sulfinpyrazone, G-32642 and G-29701 was established by a countercurrent distribution procedure [11] involving eight transfers using ethylene dichloride and various aqueous buffer solutions.† In each case the authentic drug distributed about equally between the two phases. After countercurrent distribution the partition ratios of the material in each tube were

measured by determining the concentration of the apparent drug in each tube. The total amount of drug submitted to countercurrent distribution was estimated from the amount found in tube 5 by application of the binomial expansion principle, in the manner described by Williamson and Craig [11]. The results obtained indicated that the method for sulfinpyrazone, G-32642 and G-29701 was about 90 per cent specific and that the method for G-31442 was about 70 per cent specific.

The technic of comparative distribution ratios [12] was used to assay the specificity of the method for G-32567 in urine. The partition ratios of the substance extracted from urine and plasma were compared with those of authentic G-32567 in a series of two phase systems consisting of ethylene dichloride and various aqueous buffers. The results indicated that both substances possessed the same solubility characteristics over a suitable pH range (6.5, 6.9 and 7), and they were therefore presumed to be identical.

The pKa of the various analogs was measured in aqueous buffer solutions by a spectrophotometric procedure [13], based on the differences in ultraviolet absorption of the ionized and non-ionized forms. The Kp (partition coefficient, a measure of the comparative lipid solubility) of the phenylbutazone analogs used for detailed clearance studies was estimated in the (virtually wholly) undissociated state by partitioning 5 mg. of the drugs between 200 ml. of 0.15 N hydrochloric acid and 20 ml. of a solvent mixture consisting of equal volumes of ethylene dichloride and normal heptane.

The binding to plasma proteins of the various drugs was estimated by equilibrium dialysis [8]. At plasma drug levels of 100 mg./L., phenylbutazone and all analogs tested were found to be approximately 98 per cent bound to plasma proteins in man. In the dog, the drugs were approximately (but not less than)

92 per cent bound to plasma proteins.

RESULTS

Table I lists the relevant phenylbutazone analogs studied, in descending order of pKa. The less acidic compounds (pKa 4.5 to 5.5) were found to be excreted in man only to the extent of less than 2 per cent of the administered dose in twenty-four hours and, since they are also metabolized slowly, they circulate unchanged in the plasma for prolonged periods (half life, twenty to seventy-two hours). The more acidic compounds (pKa 2.3 to 3.1) were rapidly excreted in the urine (>35 per cent in twenty-four hours) and were soon cleared from the plasma (half life, one to eight hours). In a typical experiment (see Fig. 4 [14]), a human subject was first given 600 mg. sulfinpyrazone (pKa 2.8)

* Wave length at which compound was measured; usually the absorption peak.

[†] The pH's of the buffer solutions employed were: For sulfinpyrazone, 7.3; for G-31442, 7.5; for G-32642, 5.5; and for G-29701, 5.2. Equal volumes of each phase were used, except for G-31442 for which twice the volume of the aqueous phase was employed.

TABLE 1

URINARY EXCRETION, PLASMA HALF-LIFE, URICOSURIC POTENCY OF PHENYLBUTAZONE ANALOGS IN MAN (COMPOUNDS LISTED IN DESCENDING ORDER OF PKA)

Compound	R1	R ²	R ³	K_p	рКа	Plasma Half-life (hr.)	24-hr. Urinary Excretion (% of dose)	Uricosuric Potency (mg.)
G-13838	Н	Н	CH(CH ₃) ₂	>100	5.5	72	<1	>1,200
G-15140	Cl	Cl	CH ₂ CH ₂ CH ₂ CH ₃		4.8	20	<1	1,000
G-27202 (metabolite 1, oxyphenbutazone)	ОН	Н	CH ₂ CH ₂ CH ₂ CH ₃	68	4.7	72	<2	800-1,000
G-27463	OH	OH	CH ₂ CH ₂ CH ₂ CH ₃		4.6	36		1,000
G-26924	H	H	CH2-CH=CH-CH3		4.6	36	<1	
G-32170	F	F	CH ₂ CH ₂ CH ₂ CH ₃		4.5	40	<1	
Phenylbutazone	H	H	CH ₂ CH ₂ CH ₂ CH ₃	>100	4.5	72	<1	800-1,000
G-32642	ОН	H	CH2CH2SOC6H5	0.3	3.1	1	58	100-150
G-28315 (sulfinpyrazone)	Н	Н	CH ₂ CH ₂ SOC ₆ H ₅	>100	2.8.	3	43	30- 70
G-31442	H	h	CH2CH2SO2C5H5		2.7	3	35	
G-32567	SO ₂ CH ₃	SO ₂ CH ₃		>100	2.6	1	40	100-150
G-29701	OH	Н	COCH2CH2CH2		2.3	8	41	
G-30249	ОН	Н	COCH ₂ C ₆ H ₅		2.0	3		30- 70

Note: R^1 and R^2 indicate any substitutions in the benzene rings of phenylbutazone, all in the para position except G-27463 which is meta substituted. R^3 indicates any substitutions in the butyl side chain. Kp is the partition coefficient (see Methods), an index of lipid solubility. pKa is the negative logarithm of the dissociation constant. The uricosuric potency is defined as the approximate intravenous dosage required to elicit a 100 per cent increase in $C_{uric\ acid}/C_{inulin}$. The data cited are the average of at least three experiments. Omitted from this compilation are three drugs of intermediate pKa. These are G-28231 (metabolite II), G-25671 and G-28234 (p-nitrophenylbutazone). The respective characteristics of these compounds are: pKa 4.0, 3.9, 3.2; plasma half-life twelve, three, twenty-four hours; twenty-four-hour urinary excretion 8, <3, <2 per cent of dose; uricosuric potency 150–300, 150–300, 30–100 mg.

intravenously, then, a week later, 600 mg. phenylbutazone (pKa 4.5). About 45 per cent of the sulfinpyrazone administered was recovered in the urine within twenty-four hours, most of this in the first six hours, and the drug disappeared rapidly from the plasma (half life, three hours). Phenylbutazone, on the other hand, disappeared slowly from the plasma (half life, three days) and less than 1 per cent of the dose was excreted in the urine in twenty-four hours.

The further results recorded here, of more detailed clearance studies, are limited to those obtained with representative compounds with pKa's either in the distinctly lower range

(sulfinpyrazone (G-28315), G-32567, G-32642) or distinctly higher range (phenylbutazone, G-13838). It will be noted (Table 1) that the lipid solubility of these compounds, as expressed by Kp, the partition coefficient, is of the same order of magnitude, except for G-32642. This latter analog has a more polar, hydroxyl group attached to one benzene ring and is therefore very water-soluble.

Clearance Studies in Man at Acid and Alkaline Urine pH. Results with the more acidic analogs (pKa 2.6 to 3.1): Table II summarizes the results of clearance studies with G-28315, sulfinpyrazone (pKa 2.8). When infused at a constant

TABLE II
RENAL EXCRETION OF G-28315, G-32567 AND G-32642 IN MAN, AT NORMAL (ACID) URINE PH

г .	Dru	ig Infused							
Experiment No.	Priming (mg.)	Sustain- ing (mg./min.)	Urine Volume (ml./min.)	$\begin{array}{c} P_{drug} \\ (mg./L.) \end{array}$	C _{inulin} (ml./min.)	F* _{drug} (mg./min.)	UV _{drug} (mg./min.)	UV _{drug} F* _{drug}	Net T _{drug} (mg./min.
		G-2	28315: Constan	t Infusion R	ate and Consta	nnt Plasma Dru	ig Levels		
1	400	1	3.2	43.5	136	0.12	0.73	6.1	0.61
2	400	1	4.3	29.3	97.3	0.057	0.97	17.0	0.91
3	400	1	3.1	23.3	104	0.048	0.70	14.6	0.65
		G-2	28315: Increasi	ng Infusion	Rate and Risi	ng Plasma Dru	g Levels		
4	200	1	9.0	49.0	132	0.13	0.73	5.6	0.60
	300	2	3.7	65.5	127	0.17	0.86	5.0	0.69
	300	3	4.5	98.0	130	0.26	0.92	3.5	0.66
5	300	2	9.5	56.0	123	0.14	1.41	10.1	1.27
	300	3	5.7	84.0	127	0.21	1.67	8.0	1.46
	300	4	3.2	125	125	0.31	1.52	4.9	1.21
6	600	2	2.9	72.0	104	0.15	0.62	4.1	0.47
	300	3	3.3	94.0	110	0.21	0.98	4.7	0.77
	300	4	6.8	120	114	0.27	1.43	5.3	1.16
		G-3	32567: Increasi	ng Infusion .	Rate and Risir	ng Plasma Dru	g Levels		
7	600	1.4	7.4	61.0	102	0.12	1.84	15.3	1.72
	600	2.8	5.3	130	92.7	0.24	2.28	9.5	2.04
8	600	1.4	10.7	64.5	131	0.17	2.47	14.5	2.30
	600	2.8	6.4	130	125	0.33	3.35	10.1	3.02
		G-3.	2642: Increasi	ng Infusion	Rate and Risir	ng Plasma Dru	g Levels		
9	300	1	3.4	22.0	87.9	0.039	0.69	17.7	0.65
,	300	2	6.2	54.5	108	0.12	1.20	10.0	1.08
	300	3	7.7	78.0	100	0.16	1.24	7.8	1.08
10	300	1	7.3	21.0	100	0.042	1.31	31.2	1.08
10	300	2	5.6	58.7	95.5	0.042	1.64	14.9	1.53
	300	3	6.0	64.9	100	0.11	2.41	18.5	2.28
	300	3	0.0	04.9	100	0.15	2.41	16.5	2.20

Note: Each clearance result cited is the mean of results in at least three collection periods.

* Drug filtered at the glomerulus (mg./min.). Plasma concentrations of free drug (not bound to plasma protein) calculated as 2 per cent of the respective plasma total concentrations (P_{drug}).

rate, sufficient to maintain plasma drug levels of 23 to 43 mg./L., excretion of the drug in the urine of three human subjects under the normal circumstances of acid urine pH was in the range of 0.70 to 0.97 mg./minute. The quantity excreted was invariably substantially greater than that filtered at the glomerulus, calculated on the basis of 2 per cent of the drug in the plasma not bound to protein. At plasma drug

levels of 20 to 30 mg./L., the ratio UV/F was 15 to 17, at higher plasma drug levels of 40 to 125 mg./L. the ratios were lower, but invariably >1 (3.5 to 10.1). Tubular secretion of the drug is indicated. In experiments in which the rate of drug infusion was gradually accelerated, with plasma drug levels rising to 98 to 125 mg./L., a significant further increase in drug excretion occurred in only one of three subjects. This

Table III

EFFECT OF ALKALINIZATION OF THE URINE WITH SODIUM BICARBONATE ON RENAL EXCRETION

OF G-28315, G-32567 AND G-32642 IN MAN

	Drug	g Infused	Sodium	Urine						
Experi- ment No.	Priming (mg.)	Sustain- ing (mg./min.)	Bicarbonate Infused (mEq./min.)	Volume	$\frac{P_{\rm drug}}{(mg./L.)}$	C _{inulin} (ml./min.)	F*d.ug (mg./min.)		Net T_{drug} (mg./min.)	
					G-28315					
1	400	2.0 2.0	0 2.7	12.4 17.5	66.5 82.0	135 150	0.18 0.25	2.27	2.09	6.4
2	300	1.5	0 1.8	1.5	30.0 18.0	125 123	0.075 0.044	0.64	0.58	6.4 7.6
					G-32642					
3	400	2.0	0 2.2	6.3	37.1 25.2	63.1 70.8	0.047 0.036	1.40	1.35	5.6
4	400	2.0	0 3.0	3.1	22.3 15.0	94.8 112	0.042 0.034	2.30 2.11	2.26 2.08	5.7
					G-32567					
5	400	2.0 2.0	0	13.0 7.0	21.4	101 106	0.043 0.052	2.80 2.65	2.76	6.8
6	400	2.0	0 2.2	1.7	85.0 72.3	77.2 77.7	0.13	1.84	1.71	6.8

Note: Each clearance result cited is the mean of results in at least three collection periods.

* Drug filtered at the glomerulus (mg./min.). Plasma concentrations of free drug (not bound to plasma protein) calculated as 2 per cent of the respective plasma total concentrations ($P_{\rm drug}$).

would suggest that the secretory Tm was approximated, assuming the ratio of reabsorbed to filtered drug to remain essentially unchanged.

The results of clearance studies with two other compounds of low pKa, namely G-32567 (pKa 2.6) and G-32642 (pKa 3.1) were similar. (Table II.) In each instance the drug was excreted in the urine in readily measurable quantities, considerably in excess of those filtered, indicating net tubular secretion. As the rate of infusion was increased and plasma drug levels rose, drug excretion was further augmented but some experiments again suggest that a secretory Tm was approximated.

The effects of alkalinization of the urine on renal excretion of these drugs in man were equivocal. (Table III.) In some experiments there was a small increase in per cent excretion of the drug administered, in others a slight decrease, or no change. These differences are

considered to be within the limits of experimental variation.

Renal excretion of the less acidic analogs (pKa 4.5, 5.5): Because of their very low rate of excretion in the urine in man (Table 1), satisfactory clearance measurements of phenylbutazone and of its less acidic analogs could not be made. The order of magnitude of drug excretion in attempts to carry out clearance studies is given in Table IV, which summarizes experiments with phenylbutazone and G-13838. While the amount of drug filtered at the glomerulus (calculated on the same basis of 2 per cent of the drug in plasma not bound to protein) was estimated to be about the same as in the case of sulfinpyrazone, only a few micrograms were excreted per minuteconcentrations too small for precise measurement by the methods employed. Evidently, tubular reabsorption was virtually complete. Upon alkalinization of the urine with sodium

Table IV
RENAL EXCRETION OF PHENYLBUTAZONE AND G-13838 IN MAN; EFFECT OF ALKALINIZATION OF
URINE WITH SODIUM BICARBONATE

			ORGINE TO	DOD LO	01011111011111			
Experiment No.	Period	Urine Volume (ml./min.)	P _{drug} (mg./L.)	C _{creatinine} (ml./min.)	F*drug (µg./min.)	UV _{drug} (µg./min.)	UV _{drug} F* _{drug}	Urine pH
			Prime: Phen	ylbutazone 600	mg. Intravenou.			
1	1-3	0.9	61.0	77.4	94	<2	<0.02	5.6
			NaHCO ₃	1.8 mEq./minus	te Intravenous			
	4, 5 6, 7 8	2.2 6.0 8.7	58.8 57.5 50.5	91.0 78.4 80.3	107 90 81	<3 8 14	<0.03 0.09 0.17	5.8 6.7 7.6
			Prime: (G-13838 600 mg	. Intravenous			
2	1-3	1.7	73.5	71.6	105	<4	< 0.04	6.8
		,	NaHCO ₃	2.0 mEq./minu	ite Intravenous			
	4-6	2.4	65.5	87.5	115	9	0.08	7.6

* Drug filtered at the glomerulus (mg./min.). Plasma concentrations of free drug (not bound to plasma protein) calculated as 2 per cent of the respective plasma total concentrations (P_{drug}).

bicarbonate there was a marked per cent increase in urinary excretion of the drugs but the absolute quantities remained inconsequential, and the results of alkalinization are considered indecisive.

Clearance Studies in the Dog at Acid and Alkaline Urine pH. In the dog, the various phenylbutazone analogs are bound to plasma proteins to a somewhat lesser extent than in man, 92 per cent as compared with 98 per cent, at the plasma drug levels considered here. Consequently, despite the lower glomerular filtration rates in the dogs as compared with the human subjects studied, the quantities of drug filtered at the glomerulus are substantially greater in the dog.

After infusion of compounds of lower pKa, G-28315 or G-32567, in dosages indicated in Table v, the drugs were excreted at rates of 0.5 to 0.6 mg./minute in acid urine, with UV/F ratios of 1.6 to 1.9, implying net tubular secretion. After alkalinization of the urine, in one experiment there was an equivocal increase in excretion of G-28315, to slightly over 0.7 mg./minute with a UV/F ratio of 2.0.

After infusion of compounds of higher pKa, phenylbutazone and G-13838, at rates yielding

plasma drug concentrations of roughly 50 to 150 mg./L., the drugs were excreted at rates of 0.05 to 0.11 mg./minute in acid urine. UV/F ratios were about 0.1 to 0.4, indicating net tubular reabsorption. As in man, much smaller quantities of phenylbutazone and G-13838 than of G-28315 and other low pKa compounds were eliminated in acid urine; nevertheless the amounts of phenylbutazone and G-13838 found in the urine were substantially larger in the dog than in man. How much increased filtered load, greater tubular secretion and/or less tubular reabsorption contributed to this difference in UV_{drug} in dog and man is not apparent from these clearance measurements.

After alkalinization of the urine, the excretion of phenylbutazone in the dog was markedly augmented, two- to fourteenfold, to give UV_{phenylbutazone} levels of 0.4 to 0.7 mg./minute; in two experiments UV/F ratios reached 1.3 and 1.5, implying net tubular secretion. Thus, in the dog, alkalinization of the urine brought the UV_{phenylbutazone} and UV/F_{phenylbutazone} about up to the levels observed with G-28315 and G-32567, a more striking response to alkalinization than with the latter drugs. It should be noted also that

the effect of alkalinization on $UV_{phenylbutazone}$ and $UV/F_{phenylbutazone}$ was much more pronounced in the dog than in man. These differences in response are not attributable to alterations in the filtered drug load, which was not significantly affected by alkalinization of the urine.

In a similar experiment with G-13838, the effects of alkalinization of the urine were in the same direction but less pronounced, in part because of concomitant falls in C_{creatinine} and P_{drug}.

Stop-flow Studies in the Dog. Figure 1 illustrates the results of a stop-flow study with G-28315. sulfinpyrazone (pKa 2.8). In the free flow periods before and after stasis the mean U/F_{drug}: U/P_{creatinine} ratios approximated 1.6. (Table v.) In the proximal segment of the tubules, i.e., in urine samples constituting the 75 to 100 per cent aliquots of the tubular urine, and corresponding to the general area of peak PAH secretion and uric acid reabsorption, the U/F_{drug}: U/P_{creatinine} ratios rose to 2.9. In the more distal segments the U/F_{drug}:U/P_{creatinine} ratios declined to a nadir of 0.8. These data imply maximum net secretion of G-28315 in the proximal segment of the tubules in the dog, and maximum net reabsorption in the more distal segments, at the major site of acidification of the tubular urine.

In another experiment with G-28315 the effect of alkalinization of the urine with sodium bicarbonate was observed. (Fig. 1.) In the free flow periods before stasis the mean $U/F_{\rm drug}$: $U/P_{\rm creatinine}$ ratio was 1.8. (Table v.)

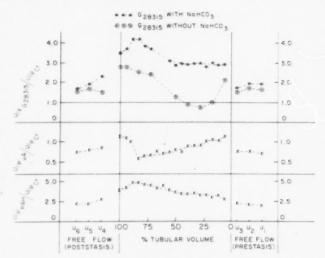


Fig. 1. Stop-flow studies in two dogs infused with G-28315 (pKa 2.8), contrasting the pattern of excretion of the drug in alkaline urine (top curve, asterisks) with that in acid urine (below, circles). In both instances peak net secretion $(U/F_{G-28315};U/P_{creatinine}\ ratios>1)$ occurs in the proximal portion of the tubules, identified as the 75 to 100 per cent aliquot of the tubular volume and the site of peak PAH secretion and peak uric acid reabsorption. (PAH secretion and uric acid reabsorption are both suppressed by G-28315, but residual activity suffices to serve as a marker). Some tubular reabsorption of drug occurs in the ordinarily acid urine of the more distal segments $(U/F_{G-28315}; U/P_{creatinine} \text{ ratios} < 1)$ but is no longer clearly demonstrable when the urine is made alkaline. See text for more complete experimental details and interpretation.

In the stop-flow periods the sinusoid curve previously obtained was no longer in evidence, the U/F_{drug} : $U/P_{\text{creatinine}}$ ratios remained consistently high. In the proximal segment the peak

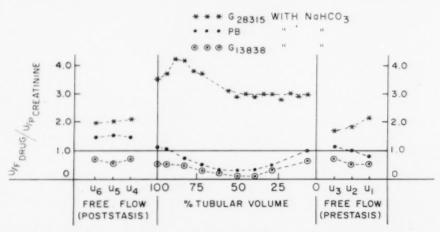


Fig. 2. Stop-flow studies in three dogs, contrasting the pattern of excretion, in alkaline urine, of G-28315 (pKa 2.8) (from Fig. 1) with that of phenylbutazone (pKa 4.5) and G-13838 (pKa 5.5). The $U/F_{\rm drug}$: $U/P_{\rm creatinine}$ ratios for phenylbutazone and G-13838 are less than 1 throughout most or all of the tubular volume, indicating net tubular reabsorption. See text for more complete experimental details and interpretation.

TABLE V

RENAL EXCRETION OF G-28315, PHENYLBUTAZONE AND G-13838 IN THE DOG; EFFECT OF ALKALINIZATION OF THE URINE WITH SODIUM BICARBONATE

	Drug	g Infused	Sodium							
Experi- ment No.	Priming (mg.)	Sustain- ing (mg./min.)	Bicarbonate Infused (mEq./min.)	Urine Volume (ml./min.)	$\begin{array}{c} P_{drug} \\ (mg./L.) \end{array}$		F* _{drug} (mg./min.)	UV _{drug} (mg./min.)	UV _{drug} F* _{drug}	Urine
				(G-28315					
1	300	1.5	0	7.3	62.9	60.7	0.31	0.50	1.6	
*	500		0	8.1	65.2	60.4	0.32	0.51	1.6	
2	300	1.5	0	18.0	71.2	64.8	0.37	0.60	1.6	6.4
			2.5	24.0	73.0	72.5	0.42	0.74	1.8	7.2
			2.5	30.0	65.6	67.5	0.36	0.72	2.0	7.6
	1			(G-32567					
3	300	2.0	0	13.2	57.3	74.4	0.34	0.61	1.8	6.4
5	300	2.0	0	10.0	54.5	49.2	0.21	0.41	1.9	6.4
	,			Phen	ylbutazone					
4	300	1.0	2.5	8.6	47.5	71.5	0.27	0.05	0.17	7.3
	500		2.5	10.6	49.0	78.1	0.31	0.07	0.24	7.6
5	600	1.0	0	12.2	76.8	76.5	0.47	0.05	0.11	6.4
			2.5	18.8	78.0	85.0	0.53	0.43	0.81	7.4
			2.5	19.4	78.8	86.5	0.55	0.54	1.0	7.6
			2.5	22.0	79.5	76.3	0.49	0.72	1.5	7.6
6	600	1.0	0	18.9	106	65.4	0.55	0.11	0.20	7.0
			2.5	23.3	140	69.9	0.78	0.29	0.37	7.2
			2.5	20.0	149	69.0	0.83	0.23	0.28	7.6
-	1000	1.0	2.5	22.0	155	69.0 20.6	0.86	0.43	0.39	6.8
7	1000	1.0	0 2.5	6.6	118 117	20.3	0.19	0.11	0.59	7.2
			2.5	12.2	116	35.9	0.33	0.44	1.3	7.6
				(G-13838					
8	600	1.0	0	14.2	73.9	54.0	0.32	0.07	0.23	7.0
0	000		2.5	11.0	61.0	42.0	0.21	0.12	0.57	7.6
			2.5	10.3	62.3	42.0	0.21	0.10	0.46	7.8

Note: Most of these data were obtained in the free flow periods of stop-flow experiments involving one kidney and have been multiplied by two for the sake of uniformity. Each clearance result cited is the mean of results in at least three collection periods.

* Drug filtered at the glomerulus (mg./min.). Plasma concentrations of free drug (not bound to plasma protein) calculated as 8 per cent of the respective plasma total concentrations (P_{drug}).

ratio reached 4.2 in this dog, declined only to about 3 distally; the pronounced fall in the $U/F_{\rm drug}$: $U/P_{\rm creatinine}$ ratios below 1 in the distal segment, observed in the acid urine of the preceding experiment, was absent. The results suggest that alkalinization of the urine diminished reabsorption of the drug from the ordinarily acid

urine of the distal tubular lumen. The distal flat curve implies that, in its passage through the distal portions of the tubule, the intraluminal fluid was either not modified at all by tubular reabsorption or secretion of G-28315, or in such proportions of both as to cause no net change.

A stop-flow experiment with G-32567, an-

other analog of lower pKa (2.6), gave free flow $U/F_{\rm drug}$: $U/P_{\rm creatinine}$ ratios before and after stasis of about 1.8 in acid urine. (Table v.) Peak net secretion, at a ratio of 2.6, occurred in the proximal tubular segment. More distally, the $U/F_{\rm drug}$: $U/P_{\rm creatinine}$ ratios declined only to 1.6; thus net tubular reabsorption, even in acid urine, was not demonstrated. The stop-flow curve for G-32567 in acid urine consequently resembled the curve for G-28315 in alkaline urine, albeit at a uniformly lower level.

In view of the slow rate of renal excretion of the phenylbutazone analogs of higher pKa in acid urine, even in the dog, stop-flow experiments without alkalinization of the urine were not attempted. In Figure 2 we have compared the results of stop-flow studies in alkaline urine with G-28315 (pKa 2.8) (from Figure 1) with those obtained with phenylbutazone (pKa 4.5) and G-13838 (pKa 5.5) at comparable plasma drug levels. In the case of phenylbutazone, the free flow U/F_{drug}: U/P_{creatinine} ratios before and after stasis were 1.0 and 1.5, respectively. (Experiment 5, Table v.) The stop-flow curve showed a broad downward sweep of the U/F_{drug}: U/P_{creatinine} ratios throughout the proximal and distal tubular segments, reaching a nadir of 0.3 well distal to the (proximal) area of peak PAH secretion. The results reflect the predominant reabsorption of phenylbutazone in its passage through the tubular lumen, even in alkaline urine. It is suggested that, with appropriate drug loads, such tubular secretion as occurs takes place in the proximal segment of the tubule. A similar stop-flow study with G-13838 gave comparable results, except that the U/F_{drug}:U/P_{ereatinine} ratios were lower throughout. Tubular secretion of G-13838 was not demonstrated.

COMMENTS

The data indicate that, under the normal circumstances of acid urine, the rate of renal excretion of phenylbutazone analogs in man is inversely related to their pKa (acidity). Thus at comparable plasma drug levels, G-28315, G-32567 and G-32642 (pKa 2.8, 2.6 and 3.1, respectively) are excreted at a rate several hundredfold greater than phenylbutazone (pKa 4.5) or G-13838 (pKa 5.5). Since all the compounds in question circulate in the blood very largely bound to plasma protein (about 98 per cent in man at the drug levels considered here), and the quantities filtered at the glomerulus

hence are uniformly small, these marked differences in the rate of renal excretion must depend upon differences in the rate of tubular secretion and/or reabsorption.

Renal clearance studies in man demonstrated that the more acidic analogs appeared in the urine in excess of the quantities filtered at the glomerulus (net secretion) whereas the reverse held for the less acidic analogs (net reabsorption). Since such over-all clearance studies do not, of course, fully differentiate the direction or extent of fluxes across the tubular cells in the several tubular segments, an effort was made to extend the analysis by dissociating (insofar as this is possible) the processes of tubular secretion from those of reabsorption, and to elucidate the respective roles of active and passive tubular transport in these processes.

Relationship of pKa to Back-Diffusion of Phenylbutazone Analogs. The fact that all these acidic drugs are so largely bound to plasma protein simplifies the analysis in one respect. Under these conditions the concentration of nonionized, readily diffusible drug in the plasma must be very low. The drug concentration in the glomerular filtrate is correspondingly low, but reabsorption of water and active secretion of drug in the proximal segment (vide seq.) soon increase the drug concentration in the intraluminal fluid, creating an "uphill" concentration gradient from peritubular fluid to tubular lumen. Such a concentration gradient argues against the possibility of simple diffusion of drug from peritubular fluid to tubular lumen, but favors diffusion from the tubular lumen into the peritubular fluid. This would suggest that the inverse ratio between pKa and the rate of renal excretion of these organic acids depends, at least in part, upon back-diffusion of the drugs in the undissociated state.

The relationships of pKa and pH relevant to such back-diffusion are given by equations (1) and (2):*

Non-ionized fraction =
$$\frac{\text{Ionized fraction}}{10^{(\text{pH-pKa})}}$$
 (1)

$$R = \frac{C_U}{C_P} = \frac{1 + 10^{(pHv - pKa)}}{1 + 10^{(pHp - pKa)}}$$
 (2)

where R is the ratio, in a steady state, of the concentration of a weak acid between urine

^{*} Both of these equations derive from the Henderson-Hasselbalch equation (see Milne, Scribner and Crawford [15] for a full discussion of the theoretic considerations involved).

(C_U) and plasma (C_P). It follows from equation (1) that in the acidified urine of the distal tubular segments, assumed to be pH 5.5 for purposes of simplicity, 50 per cent of G-13838 (pKa 5.5) and 9.1 per cent of phenylbutazone (pKa 4.5) would be in non-ionized, readily diffusible form and the drug would be largely reabsorbed, with correspondingly little excreted. In contrast, in the case of an analog of pKa 2.5, only 0.1 per cent would be undissociated, hence a much larger proportion would be excreted. Thus the *direction* of observed differences in the rate of excretion of the phenylbutazone analogs is in accord with prediction based on non-ionic back-diffusion of the drugs.

The rate of urinary excretion depends, however, not only on the proportion but also on the absolute quantity of ionized drug present at the tubular site of back-diffusion (equation 2). Excretion of the more acidic G-28315 (pKa 2.8) is several hundredfold greater than that of the less acidic phenylbutazone (pKa 4.5), for example, a much larger difference than accounted for simply by the proportion of ionized to nonionized drug alone. This discrepancy implies a higher intraluminal concentration of the more acidic drugs than of the less acidic drugs, as will be brought out later. Many other factors (Kp. rate of urine flow, plasma protein binding) affect the rate and extent of diffusion across the tubules (vide seq.).

The conventional experimental support for diffusion of compounds across the tubules is based on the demonstration of dependence upon urine pH. In the present studies the results of alkalinization of the urine in man were inconclusive. The data do suggest a proportionately larger increase in the renal excretion of phenylbutazone analogs of higher pKa (from more than two- to sevenfold in two experiments), compared to those of lower pKa (percentual variations probably within the limits of experimental error). However, the very small absolute quantities of the less acidic phenylbutazone and G-13838 excreted, even with alkalinization, make interpretation uncertain.

The reasons for these equivocal results are discernible in the implications of equations 1 and 2. It is implicit in these equations that alkalinization of the urine leads to increased ionization of weak acids, with correspondingly greater trapping in the tubular urine and enhanced excretion. The lower the pKa of the drug, however, the less would be the progressive

absolute increase in ionized drug in progressively alkalinized urine. When the drug already is virtually completely ionized at acid urine pH, any further increase in urine pH has little or no measurable effect on excretion. Thus if the pH in the ordinarily acid urine of the distal tubular segments (previously postulated to be 5.5) were now 7.5, G-13838 (pKa 5.5) would no longer be 50 per cent non-ionized at that site in the nephron but only 0.99 per cent non-ionized, a proportion which might still permit some reabsorption, depending upon Kp, urine flow rates and other conditions. Phenylbutazone (pKa 4.5) would now be 0.1 per cent instead of 9.1 per cent non-ionized. An analog of pKa 2.5, only 0.1 per cent non-ionized at pH 5.5, and very largely non-diffusible, would be but 0.001 per cent non-ionized at pH 7.5, with little or no further measurable change in excretion. These considerations, and the possibility of concomitant active tubular secretion of varying degree, limit the usefulness of the demonstration of dependence of the rate of renal excretion upon the urine pH as a test of back-diffusion.

A more convincing case for urine pH-dependence could, however, be made in the dog, in which more readily measurable amounts of the phenylbutazone analogs appear in the urine, in part because of somewhat lesser binding to plasma protein (about 92 per cent). In this species the excretion of phenylbutazone (pKa 4.5) and of G-13838 (pKa 5.5) was unequivo-cally greater at urine pH 7.6 to 7.8 than in acid urine whereas the excretion of G-28315 (pKa 2.8) was not appreciably increased, at least percentagewise, by alkalinization of the urine.

In stop-flow studies the limited back-diffusion of a more acidic analog (G-28315, pKa 2.8) that occurred at acid urine pH, in the more distal tubular segments where the urine is acidified, could be shown to be abolished by alkalinization of the urine. In the case of less acidic analogs (phenylbutazone, pKa 4.5, and G-13838, pKa 5.5), although net reabsorption of drug was markedly reduced in alkaline urine it could still be readily demonstrated in the more distal tubular segments, and perhaps occurred in the proximal as well. These responses are in accord with prediction from equations 1 and 2. If it is considered that the pH.of the plasma and the glomerular filtrate is about 7.4, and the pH of the intraluminal fluid is about 7.0 [16] until the distal tubular segments are reached where it is assumed to be pH 5.5, there is an over-all

hydrogen ion gradient from tubular urine to plasma throughout the length of the tubule, smaller in the more proximal segments, larger in the more distal segments. (For purposes of simplicity, the pH gradients across the intervening cell membranes are here ignored.) For less acidic analogs like G-13838 (pKa 5.5) or phenylbutazone (pKa 4.5) the hydrogen ion differential between pH levels of 7 and 7.4 might permit some back-diffusion of non-ionized drug throughout almost the entire length of the more proximal tubular lumen; and the drug remaining in the tubular fluid would be virtually completely reabsorbed in the more acid, distal tubular lumen. Alkalinization of the urine would be expected to reduce but not completely abolish back-diffusion in the more distal tubular fluid, now no longer acid, while reabsorption of drug in the more proximal tubular lumen would be less affected. However, in the case of analogs of lower pKa, like G-28315, appreciable backdiffusion is possible only in the acidic urine of the more distal tubular segments, and would be virtually nil throughout the nephron upon alkalinization of the urine.

The data in man and dog, then, are generally compatible with the hypothesis that the inverse relationship between pKa and rate of urinary excretion of the phenylbutazone analogs is attributable, at least in part, to non-ionic backdiffusion of the drug. In this respect the findings are in accord with a number of previously recorded observations linking the rate and extent of simple diffusion of weak acids and bases across biological membranes to their pKa and Kp (lipid solubility). These interrelationships derive from the circumstance that cell membranes exhibit a preferential permeability for lipidsoluble substances [17,18], and that molecules in the non-ionized state are more lipid-soluble, hence more readily diffusible into and out of cells, than in the ionized state.

Thus Shore, Brodie and Hogben [19,20] showed that a variety of acidic and basic drugs pass across the gastric mucosa, from plasma to gastric juice and vice versa, to a degree determined in part by their pKa. After intravenous injection to dogs with Heidenhain pouches, basic drugs appeared in the strongly acidic gastric juice at a higher concentration than in plasma, whereas the only acidic drugs that could be detected at all in gastric juice were those of relatively high pKa. Conversely, in experiments designed to measure absorption from the stom-

ach (of rats), weak acids, which are largely non-ionized at the low pH of the gastric juice, were readily absorbed whereas bases, largely present in ionized form, were poorly absorbed. When, however, the pH of the gastric contents was converted from 1 to 8 by addition of sodium bicarbonate, bases, now largely in non-ionized form, were readily absorbed; whereas acids were poorly absorbed since, being now in an ionized, poorly diffusible state, they were trapped in the alkalinized gastric contents. The transfer of acidic and basic drugs across the gastric mucosa was thus shown to occur in general accordance with prediction from the laws of simple diffusion across a biological membrane.

The hydrogen ion gradient between renal tubular fluid and plasma is, of course, much smaller than that between gastric juice and plasma but sufficient to permit diffusion of many weak acids and bases in the undissociated state, to an extent varying with the nature of the compound and the conditions of excretion [15,21]. Of the weak organic acids (our sole concern here), tubular reabsorption by non-ionic backdiffusion has been clearly demonstrated for salicylic acid, pKa 3.0 [15,22-27], phenobarbital, pKa 7.2 [28] and probenecid (p-[di-n-propylsulfamyl] benzoic acid), pKa 3.4 [29]. In man, unconjugated salicylate is excreted in acid urine at a substantially lower rate (of the same order as that of the more acidic phenylbutazone analogs) than free salicylate is filtered at the glomerulus [24], hence must be largely reabsorbed in its. passage through the tubular lumen. Probenecid is excreted in negligible amounts (of the same order as that of the less acidic phenylbutazone analogs) in acid urine, quantities less than 10 per cent of the filtered drug, and tubular reabsorption must therefore be virtually complete [29]. Upon alkalinization of the urine the output both of probenecid [29] and of unconjugated salicylate [24] is markedly augmented. Indeed the quantity excreted may then significantly exceed that filtered, thus establishing the capacity for tubular secretion of these drugs [24,29]. Since the increase in drug excretion upon alkalinization of the urine is not due to any increase in the filtered load either of probenecid or of free salicylate, it must derive from changes at the tubular level: decreased reabsorption and/or increased secretion. The evidence indicates that in both instances there is a decrease in tubular reabsorption, due to a decline in non-ionic backdiffusion as the pH of the urine rises and the

proportion of non-ionized drug correspondingly diminishes [15,24,27,29].

Thus far in this discussion we have considered only one variable, the pKa, of the many that significantly influence the rate and extent of nonionic back-diffusion of phenylbutazone analogs across the tubular cells. Another, and equally important variable is the intrinsic lipid solubility in the non-ionized state (Kp), a factor which was, however, relatively constant in all but one of the phenylbutazone analogs selected for this study. The role of lipid solubility in non-ionic back-diffusion has been clearly demonstrated for the analogs of probenecid, which all have the same pKa (3.3 or 3.4) but vary over a very wide range of Kp.* Beyer [30] showed that the renal clearance of probenecid analogs increases, in slightly acid urine, as the length of the N-alkyl substituents in the series decreases. This relationship of structure to the rate of renal clearance at acid urine pH was demonstrated by Weiner. Washington and Mudge [29] to depend upon the varying lipid solubility (Kp) of these compounds in the non-ionized state: the longer the N-alkyl chain the greater the lipid solubility, the more readily diffusible the compound, and the more non-ionic back-diffusion occurs in the tubules.

Another factor significantly decreasing back-diffusion, hence increasing excretion of drugs, is very rapid urine flow [29]. The rate of urine flow varied considerably from experiment to experiment in some of our own studies (which incidentally illustrate the point) but was usually successfully maintained within acceptable limits in any one experiment. Still another factor, which doubtless significantly affects R, the concentration ratio of equation 2, is the degree of binding to plasma proteins; this, however, was about the same, within any one species, for all the phenyl-butazone analogs here considered, at the plasma drug levels in question.

Possible Relationship of pKa to Active Tubular Secretion of Phenylbutazone Analogs. Whereas tubular reabsorption of the phenylbutazone analogs can be accounted for adequately by non-ionic back-diffusion, without necessitating the assumption of active transport, there is good reason to believe that drug accumulation in the tubular lumen, as indicated by clearance ratios >1 shown for all save G-13838 (pKa 5.5), involves active tubular secretion. It has already

been pointed out that the drug concentration gradient across the tubular cells from peritubular fluid to tubular lumen is "uphill." Moreover, phenylbutazone [31] and those of its analogs that have been studied in this regard [7,32,33] depress TmpaH and the tubular secretion of phenolsulforphthalein [10], presumably competitively. Competitive inhibition implies that the processes of tubular secretion of the phenylbutazone analogs are of limited capacity, which is indeed suggested by the clearance data insofar as this can be ascertained under conditions of concomitant reabsorption. Stop-flow studies in the dog indicate that the site of secretion of these drugs, when demonstrable, is the proximal segment of the tubule.

While the phenylbutazone analogs (like PAH. phenolsulfonphthalein, salicylate and a number of other organic acids) all appear to be actively secreted by the tubules, the present study suggests that the rate of secretion is not the same for all the analogs investigated, even at comparable plasma drug levels. Thus secretion of the analogs of lower pKa, such as G-28315, G-32642 and G-32567, was easily demonstrable by clearance measurements, drug/glomerular filtration rate ratios well in excess of 1 being readily obtained; whereas ratios > 1 were reached with phenylbutazone (pKa 4.5) only after heavy drug loads. and could not be achieved at all with G-13838 (pKa 5.5). To be sure, it is these latter drugs, of higher pKa, in which non-ionic back-diffusion in acid urine is much more pronounced, and this might mask vigorous tubular secretion in overall clearances. However, the differences persisted even after alkalinization of the urine, which virtually abolishes back-diffusion of the more acidic analogs and reduces back-diffusion of the less acidic compounds; this is shown particularly clearly by the much higher U/Fdrug: U/Pcreatinine of the more acidic analogs in stop-flow studies after alkalinization of the urine in the dog. Just how the pKa of these acidic drugs could so affect their rate of active tubular secretion is, for the present, a matter of intriguing speculation.

If there is indeed, as would appear, a general inverse relationship between the rate of active tubular secretion of the phenylbutazone analogs and their pKa, this might account in large part for the apparent discrepancy between the several hundredfold greater rate of renal excretion found for the analogs of lower pKa, as compared with that of the analogs of higher pKa. As already noted, a much smaller difference would be

^{*} For the various probenecid analogs studied, the range of Kp in a chloroform-water (0.15 M HCl) system was from <0.002 to >2,000, or a span of more than 10⁶.

predicted on the basis of non-ionic backdiffusion alone; and the amounts of the drugs filtered at the glomerulus are about the same, at least for any one species. A higher rate of tubular secretion of analogs of lower pKa, which is inferred, would increase the total concentration of drug available in the tubular lumen at the distal site of acidification of the urine, and hence the absolute amount of ionized drug excreted would be augmented: the reverse would be true of analogs of higher pKa. The combined results of these two effects of the pKa, one governing the rate of non-ionic back-diffusion, the other influencing the rate of active tubular secretion, would be to exaggerate the inverse relationship of the pKa of the drugs to the rate of their renal excretion.

Another phenomenon which may be clarified by these considerations concerns the inverse relationship previously noted [1] between the pKa of the phenylbutazone analogs and their potency as uricosuric agents; this relationship is sufficiently established to make possible prediction of uricosuric effectiveness as the molecular structure of phenylbutazone is altered [14]. If analogs of lower pKa are indeed more rapidly secreted by active tubular processes than are analogs of higher pKa, this might explain why the active reabsorption of uric acid, which seems to take place in the same (proximal) tubular segment, is more effectively inhibited by sulfinpyrazone (pKa 2.8), for example, than by phenylbutazone (pKa 4.5). The nature and precise cellular localization of this bidirectional interference, perhaps competitive, have yet to be elucidated.

In the literature, little is found concerning the comparative rates of active tubular secretion of organic acids, presumably because of the difficulties in measurement, particularly in the face of concomitant tubular reabsorption, and because most reports have dealt with individual compounds rather than with series of related analogs. The subject was considered at some length in 1943 by Fisher, Troast, Waterhouse and Shannon [34] in their study of sulfanilamide analogs. Noting both concordances and discordances in the inverse relationship between pKa and rate of renal excretion of sulfanilamide analogs, these investigators emphasized the importance of two factors, the strength of acidic groups of the molecule, and the molecular configuration of the compounds as a whole. Shortly thereafter Smith et al. [35,36], in their classic

study of substituted hippuric acid derivatives, found no simple relationship between the variation in the clearance of these compounds (about as high as C_{diodrast} in most instances) and their pKa, for the most part in the range 3.6 to 4.2. It would be of interest to analyze these two series of analogs more precisely in terms of lipid solubility, protein binding, concomitant reabsorption and the like.

Differences in extraction ratios, which may be marked, presumably contribute to differences in the rate of secretion. Tubular secretion may, of course, involve distinct transport systems, operating at different rates, for different classes of compounds. Species differences between man and dog in the rate of active tubular secretion of organic acids are well recognized, for example

in respect to PAH.

Concluding Remarks. Thus far, stress has been placed in this discussion on the one general mechanism of renal regulation of organic acid excretion with which we are here principally concerned but, for proper perspective, others, equally important, should be mentioned. Thus, when renal conservation of weak acids is in order, as in the case of a variety of metabolically derived organic acids, tubular transport mechanisms are available for accelerated reabsorption of the filtered and/or secreted compounds. Under these circumstances, non-ionic backdiffusion apparently plays a minor if any role. Uric acid (pKa 5.4), for example, is filtered, secreted by active processes (in the mammal also [6,37,38]), then very largely reabsorbed by the tubules; this last is accomplished by active processes for the most part but also apparently by back-diffusion in small measure, to judge by some urine pH-dependency [26].

Renal conservation of components of the Krebs tricarboxylic acid cycle is accomplished by retention and utilization within the cells of the tubules. Thus infusion of citrate, α-ketoglutarate or succinate leads to net tubular secretion of malic acid, a subsequent intermediate in the tricarboxylic acid cycle [39–41]. It is interesting to note that when L-malic acid, the natural isomer, is itself infused, the filtered malate is conserved by active tubular reabsorption; however, when p-malic acid, the unnatural and metabolically useless isomer, is infused, its elimination is hastened by active tubular secretion [42,43]. The natural L-isomers of amino acids are actively reabsorbed whereas tubular reabsorption of p-isomers of many amino acids seems to be limited largely or entirely to back-diffusion [44,45].

Phenylbutazone and its congeners are, of course, compounds foreign to the body economy, not to be conserved but rather to be eliminated as rapidly as possible. The devices brought into play to accomplish this probably are much the same for all acidic and basic drugs. Nevertheless, in view of the variety of such devices, renal and extrarenal, and the structural diversity of the compounds dealt with, the factors affecting the rate of urinary excretion must be many and complex. Obviously, the marked differences in the rate of renal excretion of compounds so varied in structure as salicylate and probenecid cannot be attributed solely to the slight differences in their pKa's (3.0 and 3.4, respectively) but also to their diverse lipid solubility and other factors. On the other hand, the marked differences in the rate of renal excretion of phenol red and Diodrast,® which cannot be explained altogether by differences in binding to plasma proteins [46], might be related, in part, to their widely disparate pKa's (7.4 and 2.7, respectively).

The experience with the phenylbutazone analogs would suggest that, as a rule, foreign compounds that are stronger organic acids of lower pKa can be readily excreted as such by the kidneys. This is effected in part by glomerular filtration, which is, however, of limited efficacy in many instances because of a high degree of binding of the drugs to plasma proteins; it is greatly facilitated by the presence of tubular transport systems for rapid active secretion; and is aided by the absence of tubular transport systems for active reabsorption, thus permitting prompt excretion of the ionized drug in the acidified urine since non-ionic back-diffusion is of small proportions. The rapidity of renal excretion of such acidic drugs of lower pKa limits the role of metabolic pathways for conjugation and degradation, at least quantitatively. In the case of foreign compounds that are weak organic acids of higher pKa, on the other hand, elimination by the kidneys seems to be hampered at every turn. Not only is glomerular filtration of the compound apt to be just as slow as in the previous instance but (at least for the phenylbutazone analogs) secretion by the tubular transport systems also seems to be more sluggish, and reabsorption, even in the absence of tubular transport systems, is more rapid and complete if the compounds are at all lipid-soluble. Under these circumstances the various metabolic pathways for conjugation and degradation to more readily excretable compounds [47,48] doubtless play a much more important quantitative role in riddance. Weak organic acids of intermediate pKa, while omitted from consideration here in order not to complicate further the issues involved, apparently evoke both excretory and metabolic mechanisms, in variable degree.

It is interesting to note in this connection that, as Quick [49] pointed out in 1932, a variety of foreign compounds that are weakly acidic, and slowly excreted, are conjugated to form stronger acids which are then rapidly eliminated by the kidney. Such conjugates as glucosiduronic acids, arylsulfuric acids, hippuric acids and mercapturic acids are, in fact, relatively strong acids, with pKa's generally between 3 and 4. Additional data in support of this "increased acidity" hypothesis of detoxication of weak acids and bases, by the formation of more strongly acidic conjugates that are more rapidly excreted (by the mechanisms herein described), are given by Williams [47].

It may not be amiss before concluding to suggest certain practical implications of these observations. As has already been indicated [19, 20], appreciation of the role of the pKa in the diffusion of weakly acidic or basic drugs across the gastric mucosa may prove useful in anticipating the rate and extent of their influx and efflux from the gut. Similarly, the present study of the inverse relationship between pKa and rate of renal excretion of the phenylbutazone analogs may facilitate prediction of the plasma half-life and excretory rate of other acidic drugs that may be under consideration for synthesis; at least, the pKa seems to be one of the several important factors involved. Elucidation of the inverse relationship between pKa and uricosuric potency of the phenylbutazone analogs has already proved useful in respect to synthesis of new uricosuric agents [14].

SUMMARY

Renal clearance studies, in man and dog, were made of a series of phenylbutazone analogs, related in structure and similar in lipid solubility and binding to plasma protein (about 98 per cent in man) but differing widely in acidity (range of pKa, 2.0 to 5.5). It was found that the rate of renal excretion of these compounds varied inversely with their pKa. The more acidic analogs were rapidly excreted in man under the normal circumstances of acid urine (net secretion

in clearance studies), the less acidic analogs were very slowly excreted (net reabsorption). Stop-flow studies in the dog revealed that peak secretion of the drugs, when demonstrable, occurred in the proximal tubular segment. Reabsorption occurred chiefly from the acidified urine of the more distal tubular segments, but the less acidic analogs may be appreciably reabsorbed more proximally also.

Analysis of the data on the rate of excretion of the phenylbutazone analogs in acid and alkaline urine indicates that tubular reabsorption varies directly with the pKa of the compound and therefore is attributable to non-ionic backdiffusion. Tubular secretion of the phenylbutazone analogs, on the other hand, occurred under conditions necessitating the assumption of active tubular transport. While active tubular secretion could not be measured directly by the technics employed, particularly in the presence of concomitant tubular reabsorption, evidence is presented to suggest that the phenylbutazone analogs of lower pKa were secreted by the tubules more rapidly than were the compounds of higher pKa. It is therefore inferred that the inverse relationship between the acidity and the rate of renal excretion of the phenylbutazone analogs may represent the summation of what seem to be two distinct effects of the pKa: one directly relating pKa to non-ionic back-diffusion, the other, apparently, inversely relating pKa to the rate of active tubular secretion. It is further suggested that the effect of pKa on the rate of active tubular secretion may be associated also with the previously described inverse relationship of pKa to uricosuric potency of the phenylbutazone analogs.

Whereas metabolically derived organic acids ordinarily are effectively conserved, by active processes of reabsorption or retention by the tubule cells, organic acids foreign to the body economy are gotten rid of by renal processes which vary in efficacy, depending in part upon the pKa of the compound. Acidic compounds of lower pKa are eliminated expeditiously, by vigorous active tubular secretion and such filtration as is permitted by the degree of binding to plasma proteins; there is no active tubular reabsorption; and, even if readily lipid-soluble, little non-ionic back-diffusion. Acidic compounds of higher pKa are eliminated at a slower rate. Active tubular secretion seems to be more sluggish; filtration is limited by the degree of plasma protein binding; and tubular reabsorption is more or less complete (if the compound is lipid-soluble) by non-ionic back-diffusion. Elimination of such drugs is facilitated by metabolic conversion to more readily excreted conjugates or degradation products. Acidic compounds of intermediate pKa are variably excreted as such and as metabolites in the urine. The relationship of these renal mechanisms to the "increased acidity" hypothesis of detoxication of organic acids by conjugation to form compounds of lower pKa, hence more rapidly excreted, is pointed out.

Appreciation of such structure-excretion relationships may prove to be helpful in anticipating the rate of renal excretion and the plasma half-life of newly synthesized acidic and basic drugs.

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REFERENCES

- Burns, J. J., Yü, T. F., Dayton, P., Berger, L., Gutman, A. B. and Brodie, B. B. Relationship between pKa and uricosuric activity in phenylbutazone analogues. *Nature*, *London*, 182: 1162, 1958.
- DAYTON, P. G., BERGER, L., YÜ, T. F., SICAM, L. E., LANDRAU, M. A., GUTMAN, A. B. and BURNS, J. J. Relationship between pKa and renal excretion of various phenylbutazone analogues. Fed. Proc., 18: 382, 1959.
- SIROTA, J. H., YÜ, T. F. and GUTMAN, A. B. Effect of benemid (p-[di-n-propyl-sulfamyl]-benzoic acid) on urate clearance and other discrete renal functions in gouty subjects. *J. Clin. Invest.*, 31: 692, 1952.
- MALVIN, R. L., SULLIVAN, L. P. and WILDE, W. S. Stop flow analysis of renal tubule localization. Physiologist, 1: 58, 1957.
- Kessler, R. H., Hierholzer, K. and Gurd, R. S. Localization of urate transport in the nephron of mongrel and Dalmatian dog kidney. Am. J. Physiol., 197: 601, 1959.
- Yü, T. F., Berger, L., Kupfer, S. and Gutman, A. B. Tubular secretion of urate in the dog. Am. J. Physiol., in press.
- Burns, J. J., Yü, T. F., RITTERBAND, A., PEREL, J. M., GUTMAN, A. B. and BRODIE, B. B. A potent new uricosuric agent, the sulfoxide metabolite of the phenylbutazone analogue, G-25671. J. Pharmacol. & Exper. Therap., 119: 418, 1957.
- 8. Burns, J. J., Rose, R. K., Chenkin, T., Goldman, A., Schulert, A. and Brodie, B. B. The physiological disposition of phenylbutazone (butazolidine) in man and a method for its estimation in biological material. J. Pharmacol. & Exper. Therap., 109: 346, 1953.

- Burns, J. J., Rose, R. K., Goodwin, S., Reichen-Thal, J., Horning, E. C. and Brodie, B. B. The metabolic fate of phenylbutazone (butazolidine) in man. J. Pharmacol. & Exper. Therap., 113: 481, 1955.
- Brodie, B. B., Yü, T. F., Burns, J. J., Chenkin, T., Paton, B. C., Stelle, J. M. and Gutman, A. B. Observations on G-25671, a phenylbutazone analogue (4-(phenylthioethyl)-1,2-diphenyl 3,5pyrazolidinedione). Proc. Exper. Biol. & Med., 86: 884, 1954.

11. WILLIAMSON, B. and CRAIG, L. C. Identification of small amounts of organic compounds by distribution studies. v. Calculations of theoretical curves.

J. Biol. Chem., 168: 687, 1947.

 Brodie, B. B., Udenfriend, S. and Baer, J. E. The estimation of basic organic compounds in biological material. I. General principles. J. Biol. Chem., 168: 299, 1947.

FLEXSER, L. A., HAMMETT, L. P. and DINGWALL, A.
 The determination of ionization by ultraviolet spectrophotometry: its validity and its application to the measurement of the strength of very weak bases. J. Am. Chem. Soc., 57: 2103, 1935.

 Burns, J. J., Yü, T. F., Dayton, P. G., Gutman, A. B. and Brodie, B. B. Biochemical pharmacological considerations of phenylbutazone and its analogues. Ann. New York Acad. Sc., 86: 253, 1960.

- MILNE, M. D., SCRIBNER, B. H. and CRAWFORD, M. A. Non-ionic diffusion and the excretion of weak acids and bases. Am. J. Med., 24: 709, 1958.
- GOTTSCHALK, C. W., LASSITER, W. E. and MYLLE, M. Localization of urine acidification in the mammalian kidney. Am. J. Physiol., 198: 581, 1960.
- DAVSON, H. and DANIELLI, J. F. The Permeability of Natural Membranes. Cambridge, 1942. Cambridge University Press.
- Danielli, J. F. The cell surface and cell physiology. In: Cytology and Cell Physiology, 2nd ed. Edited by Bourne, G. New York, 1951. Oxford University Press.
- Shore, P. A., Brodie, B. B. and Hogben, C. A. M.
 The gastric secretion of drugs: a pH partition hypothesis. J. Pharmacol. & Exper. Therap., 119: 361, 1957.
- 20. Brodie, B. B. and Hogben, C. A. M. Some physicochemical factors in drug action. *J. Pharm. & Pharmacol.*, 9: 345, 1957.
- Orloff, J. and Berliner, R. W. The mechanism of the excretion of ammonia in the dog. J. Clin. Invest., 35: 223, 1956.
- 22. Smith, P. K. Certain aspects of the pharmacology of the salicylates. *Pharmacol. Rev.*, 1: 353, 1949.
- Berliner, R. W. The kidney. Ann. Rev. Physiol., 16: 269, 1954.
- GUTMAN, A. B., YÜ, T. F. and SIROTA, J. H. A study, by simultaneous clearance techniques, of salicylate excretion in man. Effect of alkalinization of the urine by bicarbonate administration; effect of probenecid. J. Clin. Invest., 34: 711, 1955.
- MACPHERSON, C. R., MILNE, M. D. and EVANS, B. M. The excretion of salicylate. Brit. J. Pharmacol., 10: 484, 1955.
- 26. Yü, T. F. and Gutman, A. B. Studies of the paradoxical effects of salicylate in low, intermediate and

- high dosage on the renal mechanisms for excretion of urate in man. J. Clin. Invest., 38: 1298, 1959.
- WEINER, I. M., WASHINGTON, J. A., II, and MUDGE, G. H. Studies on the renal excretion of salicylate in the dog. *Bull. Johns Hopkins Hosp.*, 105: 284, 1959.
- WADDELL, W. J. and BUTLER, T. C. The distribution and excretion of phenobarbital. J. Clin. Invest., 36: 1217, 1957.
- Weiner, I. M., Washington, J. A., II and Mudge, G. H. On the mechanism of action of probenecid on renal tubular secretion. *Bull. Johns Hopkins Hosp.*, 106: 333, 1960.
- BEYER, K. H. Factors basic to the development of useful inhibitors of renal transport mechanisms. Arch. internat. pharmacodyn., 98: 97, 1954.
- YÜ, T. F., SIROTA, J. H. and GUTMAN, A. B. Effect of phenylbutazone (3,5 dioxo-1,2-diphenyl-4-nbutylpyrazolidine) on renal clearance of urate and other discrete renal functions in gouty subjects. J. Clin. Invest., 32: 1121, 1953.
- YÜ, T. F., PATON, B. C., CHENKIN, T., BURNS, J. J., BRODIE, B. B. and GUTMAN, A. B. Effect of a phenylbutazone analog (4-[phenylthioethyl]-1,2diphenyl 3,5-pyrazolidinedione) on urate clearance and other discrete renal functions in gouty subjects. Evaluation as uricosuric agent. J. Clin. Invest., 35: 374, 1956.
- YÜ, T. F., BURNS, J. J., DAYTON, P. G., GUTMAN, A. B. and BRODIE, B. B. A p-nitro analogue of phenylbutazone possessing potent antirheumatic, sodium retaining and uricosuric properties. J. Pharmacol. & Exper. Therap., 126: 185, 1959.
- 34. FISHER, S. H., TROAST, L., WATERHOUSE, A. and SHANNON, J. A. The relation between chemical structure and physiological disposition of a series of substances allied to sulfanilamide. J. Pharmacol. & Exper. Therap., 79: 373, 1943.
- SMITH, H. W., FINKELSTEIN, N., ALIMINOSA, L., CRAWFORD, B. and GRABER, M. The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. J. Clin. Invest., 24: 388, 1945.
- SMITH, H. W. The Kidney: Structure and Function in Health and Disease. New York, 1951. Oxford University Press.
- POULSEN, H. and PRAETORIUS, E. Tubular excretion of uric acid in rabbits. Acta pharmacol. et toxicol., 10: 371, 1954.
- GUTMAN, A. B., YÜ, T. F. and BERGER, L. Tubular secretion of urate in man. J. Clin. Invest., 38: 1778, 1959.
- Craig, J. W., Miller, M., Owens, J. E. and Woodward, H., Jr. Renal malic acid metabolism in vivo. Fed. Proc., 12: 29, 1953.
- VISHWAKARMA, P. Excretion of malic acid in relation to tricarboxylic acid cycle in kidney. Fed. Proc. 16: 132, 1957.
- LOTSPEICH, W. D. Kidney, water and electrolyte metabolism. Ann. Rev. Physiol., 20: 339, 1958.
- VISHWAKARMA, P. and LOTSPEICH, W. D. Excretion of L-malic acid in the chicken. Am. J. Physiol., 198: 819, 1960.
- VISHWAKARMA, P. and LOTSPEICH, W. D. Excretion of p-malic acid in the chicken. Am. J. Physiol., 198: 824, 1960.

- DOOLAN, P. D., HARPER, H. A., HUTCHIN, M. E. and SHREEVE, W. W. The renal tubular response to amino acid loading. J. Clin. Invest., 34: 1247, 1955.
- DOOLAN, P. D., HARPER, H. A., HUTCHIN, M. E. and ALPEN, E. L. Renal clearance of lysine in cystinuria. Am. J. Med., 23: 416, 1957.
- OCHWADT, B. K. and PITTS, R. F. Disparity between phenol red and diodrast clearances in the dog. Am. J. Physiol., 187: 318, 1956.
- 47. WILLIAMS, R. T. Detoxication Mechanisms. The
- Metabolism and Detoxication of Drugs, Toxic Substances and Other Organic Compounds, 2nd ed., New York, 1959. John Wiley & Sons.
- BRODIE, B. B., GILLETTE, J. R. and LADU, B. N. Enzymatic metabolism of drugs and other foreign compounds. Ann. Rev. Biochem., 27: 427, 1958.
- QUICK, J. A. The relationship between chemical structure and physiological response. II. The conjugation of hydroxy and methoxybenzoic acids. J. Biol. Chem., 97: 403, 1932.

Nucleic Acids and Cancer*

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TUCLEIC acids have recently acquired a key role in cell physiology and biochemistry because of the recognition of their unique ability to transfer information. This transfer of information or coding is a consequence of the specific chemical structure of the nucleic acid, and much of the current exciting and significant research work in this field is being directed toward an understanding of the relationship between chemical configuration and biological specificity. The cancer cell (a term used in this text to indicate all forms of malignant tumors) possesses the biological property of uncontrolled growth in the host. It is this property which allows the cell to manifest itself by invasion and metastases. Since uncontrolled growth is an hereditary property of the cancer cell, it is possible to consider the nature of this cell in terms of an alteration in the normal transfer of information at the subcellular level. With this in mind it is necessary to develop some general concepts of cell structure and function. These concepts will be oriented toward the nucleic acids, and it is within the framework of nucleic acids and information transfer that carcinogenesis and chemotherapy will be considered.

This review will be divided into four sections:
(1) the relationship of nucleic acids to cell

structure and function; (2) general mechanisms of cell differentiation, cell division and carcinogenesis; (3) specific agents in carcinogenesis; and (4) chemical agents for therapy. With such a panoramic view of the relationship of the nucleic acids to the cancer cell it is obvious that only superficial coverage can be presented in any one area. Therefore many of the references cited are review articles which serve to orient as well as to provide further references to the experimental data. Those who work in the specific areas under discussion will realize that some of the concepts and relationships expressed here lack definitive experimental support. However, it seemed profitable to include these with qualifications in order to delineate better the extent of the possible relationships of nucleic acids to cancer.

THE RELATIONSHIP OF NUCLEIC ACIDS TO CELL STRUCTURE AND FUNCTION

The development of knowledge relating nucleic acids to cell structure and to the problem of transfer of information has been possible because of the pooling of knowledge from different disciplines. It is possible to consider this general area from a biological, a morphological and a biochemical viewpoint.

Biological:	Genes -) → Enzymes (or structural
Morphological	: Chromosomes -	*	proteins) → Soluble and insoluble
Biochemical:	DNA‡ -	reticulum → RNA	proteins → Protein

[‡] The following abbreviations have been used: DNA, deoxyribonucleic acid; DNA-ase, deoxyribonuclease; RNA, ribonucleic acid; RNA-ase, ribonuclease; ATP, adenosine triphosphate; AMP, adenosine monophosphate; GTP, guanosine triphosphate; GMP, guanosine monophosphate; P-P, inorganic pyrophosphate.

Genes, Chromosomes and DNA. The equating of genes, chromosomal material and deoxyribonucleic acid (DNA) has led to a rapid advance in concepts of biological organization. The concept of a gene as a unit of inheritance,

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Fig. 1. Components of nucleic acids.

transmitted in germ cells, and controlling the development of an hereditary characteristic was well developed by 1910. The experiments of Morgan [1] and others produced evidence for the linkage and the linear arrangement of genes on chromosomes, and led to the extensive chromosomal mapping of genetic loci in *Drosophila*. Proof of the relationship of genes to chromosomes was offered when genetic deficiencies were found to correspond to cytological deletions of areas of chromosomes [2].

The presence of deoxyribonucleic acid in chromosomal material of sperm and somatic cells was demonstrated by chemical and cytochemical means [3] and this led to the hypothesis that DNA was the genetic material. The discovery of the biological phenomenon of "transformation" provided direct evidence for this hypothesis. Transformation describes a process whereby new genetic material is introduced into microorganisms. Transformation of avirulent pneumococci into a virulent form was accomplished by Griffith [4] in 1928 by the addition of heat-killed virulent pneumococci to live avirulent forms. Since the new virulent strain would produce virulent progeny, it was considered that the genetic constitution of the avirulent organism had been altered. The transforming principle was purified and found to possess all the properties of highly polymerized DNA [5]. It is now possible to introduce into an organism genetic material or DNA which will allow the organism to synthesize an enzyme which it was previously unable to make. More recently the phenomenon of "transduction" was

described by Zinder and Lederberg [6], by which hereditary characteristics are transferred from one microorganism to another via a bacteriophage or a virus the major element of which is DNA. Thus transformation [7] and transduction [8] represent processes by which new genetic material is introduced into a microorganism, and there is now excellent evidence that this material is DNA. Attempts have been made by column chromatographic technics to separate specific genetic markers from the total DNA of a microorganism. Some purification of biologically active DNA fractions has been obtained [9].

Since the genetic information must be present in the DNA molecule, coded in some manner, much experimental work has been carried out to elucidate the structure of this molecule as well as that of ribonucleic acid (RNA). DNA and RNA are polymers made up of nucleotide sub-units. Each nucleotide contains a base (Figs. 1 and 2), either a purine (adenine or guanine) or pyrimidine (cytosine and thymine in DNA and cytosine and uracil in RNA), linked to a five carbon sugar, deoxyribose or ribose, which has an esterified phosphate group at the 5' position. DNA is a polymer of deoxyribonucleotides linked by phosphodiester bonds between the 3' and 5' carbons of the deoxyribose of adjacent nucleotides. RNA contains the same phosphodiester linkage. The double helical structure of DNA (Fig. 3) was proposed by Watson and Crick [10,11] on the basis of x-ray diffraction studies, chemical analysis of base composition, physical-chemical studies of viscosity, and other evidence [12]. The double

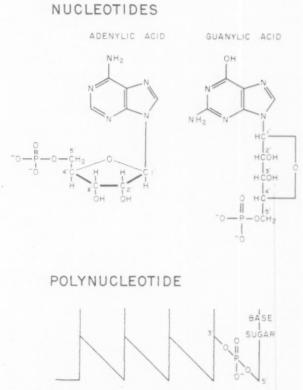


Fig. 2. Structure of nucleotides and a polynucleotide. The ribose of adenylic acid is represented in the ring form, that of guanylic acid in the straight chain form.

helix is formed by two strands of polymerized nucleotides which run antiparallel and which are linked together by hydrogen bonds between bases of the first chain and bases of the second chain. In this structure, because of the requirements for hydrogen bonding, adenine can pair only with thymine, and guanine only with cytosine. The double helix then has two complementary polynucleotide chains each one of which can direct the de novo synthesis of its mate. Experimental evidence seems to support this ingenious hypothesis concerning the structure of DNA and the replication of DNA. Each of the strands of the double helix directs the synthesis of a new strand, and the daughter cells receive a DNA molecule containing one of the original strands and one which was newly synthesized [13]. Support for the self-replication also comes from the work of Kornberg et al. [14] in which a synthetic polynucleotide containing only the nucleotides deoxyadenylic acid and thymidylic acid will catalyze the synthesis in vitro of similar molecules which contain only those two nucleotides, even though all four nucleotides are present.

Although knowledge of the genetic unit is still

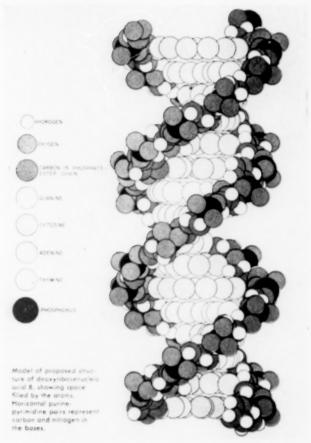


Fig. 3. A model of the structure proposed by Watson and Crick of deoxyribonucleic acid. The double helix, with the sugar-phosphate-sugar-etc. backbones runs vertically, while the purines hydrogen bonded to the pyrimidines are shown in horizontal layers. (From: Hamilton, L. O. Cancer therapy and the structure of DNA. Bull. Cancer Progr., 5: 159, 1955.)

rudimentary, the concept that one gene controls the synthesis of one enzyme or protein is generally accepted. Since the specificity of a protein resides in its amino acid sequence, that is the order of specific amino acids linked together by peptide bonds, one would presume that the alignment of amino acids in the protein would be determined by the genetic unit. The genetic unit then must contain information which is coded by the arrangement of the four bases. (Fig. 1.) A code in which three purine or pyrimidine nucleotides, arranged in a specific sequence, will determine the alignment of one amino acid, and in which only twenty amino acids can be coded has been proposed [15]. This formulation, which requires three nucleotide pairs for each amino acid, means that in one genetic locus there must be a large number of such pairs. This is in keeping with the observation of as many as forty-seven different mutations in a single genetic locus [16]. Eventually, the fine structure of the gene may be described in terms of the sequence of nucleotides, and it is possible that this sequence will be shown to correspond to the sequence of amino acids in the protein controlled by this gene. This concept of gene mutation which results in an alteration of amino acid sequence has already been demonstrated in the hereditary disease, sickle cell anemia. The abnormal sickle cell hemoglobin differs from normal hemoglobin in a portion of the globin molecule in which the amino acid valine replaces glutamic acid [17]. In hemoglobin C, lysine replaces glutamic acid at the same site [17].

Evidence now exists which suggests that the level of specific enzymes or proteins may be controlled genetically. Some diseases which are known to be due to genetic deficiencies are recessive, that is, the disease is not manifest unless the subject inherits the deficiency from both parents. In this case the two equivalent genetic loci are both defective, a homozygous state which results in inability to produce a specific enzyme, and consequently the disease is manifest. In the heterozygous state only one of the two chromosomes which bear this genetic locus is deficient, and the disease is not manifest. It has been shown that the level of enzyme present in heterozygotes may be approximately half that of the normal [18,19]. This suggests that with half of the full genetic complement, only half of the enzyme-forming systems is synthesized, and therefore the enzyme level in the cell is half of the normal value.

Enzyme-Forming Systems, Endoplasmic Reticulum and RNA. Protein synthesis in the absence of nuclear material or DNA can be observed in several cells such as the reticulocyte, and enucleated acetabularia and ameba [20]. Although synthesis of protein can occur in the nucleus and in mitochondria, the major synthesis takes place in the cytoplasm and requires soluble enzymes and ribonucleoprotein granules [21-25]. These granules, which are present in all species [24], are approximately 150 Å in diameter and have an average sedimentation coefficient of 80-S. They contain 30 to 50 per cent RNA, the remainder is protein. They are visible by the electron microscope and exist either free in the cytoplasm or associated with the complex membrane structures which are found especially in cells which synthesize protein for export out

of the cell. Electron microscopists have termed the cytoplasmic structures consisting of membranes and ribonucleoprotein granules the endoplasmic reticulum [26]. Biochemists have isolated this material by differential ultracentrifugation and identified it as the microsomal fraction [27]. Biochemical evidence suggests that the microsomes are the major site of polypeptide and protein synthesis and that the RNA in the ribonucleoprotein granule contains the specific information for alignment of the amino acids. In this manner a specific RNA molecule would act as a template for the production of many molecules of a specific protein. Although there is as yet no direct evidence that RNA from animal or bacterial ribonucleoprotein granules contains specific information, there is excellent evidence that RNA from tobacco mosaic virus (TMV) is itself infectious and that it determines the type of protein which is found in the virus progeny [28]. It has been possible to alter this infectious RNA chemically and to show that the virus progeny which contain RNA and protein are different [29]. Also some evidence has been presented to show that the nucleotide sequence [30] as well as the amino acid composition of two strains of virus differ.

Obviously the transfer of information from DNA to RNA in the cell is of extreme importance. As yet there is only fragmentary evidence of the nature of such transfer. This evidence indicates that there is rapid synthesis of RNA in the nucleus [31], that there is RNA synthesis on chromosomal material [32], at specific loci on chromosomes (puffs) [33], and that after the transfer of nuclei containing isotopically labeled RNA to unlabeled cells, the isotope moves into the cytoplasm [34].

Enzymes and Structural Proteins. The development of methods for the stepwise degradation of polypeptides and proteins [35] has solidified the concept that the species and the functional specificity of an enzyme or structural protein reside in the sequence of its amino acids [36–38]. A mechanism by which proteins are synthesized from amino acids is gradually emerging from the work of several investigators [23,24] and this mechanism may account for the specific sequence of amino acids. The process of protein biosynthesis is visualized at present in four stages:

Amino acid activation [23,24]: Energy is required for the synthesis of a peptide bond and this is supplied by the reaction of each amino acid

with adenosine triphosphate (ATP) to form a high energy amino acid-adenylic acid (AMP) complex which is tightly bound to the enzyme.

$$\begin{array}{c|c} O & NH_2 \\ & \parallel & \mid \\ ATP + HOC - C - R_1 + Enzyme \\ & O & NH_2 \\ & \parallel & \mid \\ & \rightleftarrows AMP - O - C - C - C - R_1 - Enzyme + P - P \end{array}$$

For each amino acid there is a different activating enzyme.

Amino acid transfer to low molecular weight RNA (transfer RNA or RNA_T) [23,24]:

The specific amino acid is transferred to a specific RNA which is of low molecular weight, 15,000 to 50,000. There appears to be a different type of RNA molecule for each amino acid. The amino acid is linked to the 2' or 3' hydroxyl of the terminal adenylic acid of the RNA.

Transfer of amino acid from low molecular weight RNA to the ribonucleoprotein granule or microsome [23,24]:

Amino Acid
$$-RNA_T + Ribonucleoprotein Granule + GTP + ATP \rightarrow Amino Acid $-Ribonucleoprotein Granule + RNAT + ? GMP$$$

This reaction allows the transfer of a series of amino acids to presumably specific sites on the ribonucleoprotein granule or enzyme-forming system. The amino acids are then linked together to form the peptide bonds of a specific protein. Although this phase is not well worked out, one would presume that there are some granules upon which aldolase is synthesized and others upon which globin is synthesized and others upon which globin is synthesized. The RNA present in the ribonucleoprotein granules differs from the amino acid transfer RNA in that it is of high molecular weight, in the order of one million.

Release and possible folding of protein:

Protein—Ribonucleoprotein Granule →
Protein + Ribonucleoprotein Granule

It is possible to see how, with this enzymatic machinery, amino acids can be aligned in specific sequences depending entirely upon the information available in the template or RNA of the ribonucleoprotein granule. Control mechanisms must be present in the cell to determine the number of each of the specific enzyme-

forming systems or ribonucleoprotein particles as well as the amount of enzyme synthesized. This problem will be considered in connection with differentiation.

The general outline of the chemical mechanisms for the transfer of information within a cell has thus recently become more clear. Three concepts are basic to the understanding of this process: the identification of DNA as the genetic material, the role of RNA as the template most likely responsible for the specific alignment of the amino acids in the process of protein synthesis, and finally the specific amino acid sequences which are characteristic of each different protein and are determined by the genetic structure. These concepts form the basis of the subsequent consideration of the processes of differentiation and carcinogenesis.

GENERAL MECHANISMS OF CELL DIFFERENTIATION, CELL DIVISION AND CARCINOGENESIS

Cell Differentiation. Any definition of differentiation is based upon the alteration of molecular populations within a cell as a function of time and it is this alteration which leads to the acquisition of specialized function. This implies that chemical changes may have occurred in the cell long before there are any morphological changes. One can consider that any stage of cell division beyond the fertilized ovum represents cell differentiation, and it is now becoming evident that even within a single ovum there is a form of cytoplasmic differentiation reflected by an unequal distribution of subcellular particles [20]. This distribution is thought to be the explanation for the initial differentiation which occurs in the blastula with the first cell division. As is stressed in many reviews [38-43], differentiation then must be considered as an alteration not just of morphology but of chemical structure—a problem of molecular ecology.

The structures which are of primary interest in a consideration of differentiation are the genes and the enzyme-forming systems, that is the ribonucleoprotein granules, which determine as previously outlined the enzymes or structural proteins which are the end products. It is obvious for example that in the evolution of the muscle cell the synthesis of the major proteins, actin and myosin, must be associated with the appearance of functional genes and enzyme-forming systems related to these proteins. Similarly, in the erythroblast or some more differentiated cell of this line, one would

expect to find functional genes and enzymeforming systems related to hemoglobin synthesis. Thus it seems reasonable that the process of differentiation must involve the DNA, RNA, protein concepts outlined in the first section.

To simplify a consideration of differentiation, it is possible to divide cellular material into two parts: machinery for cell division and machinery for specialized function [44]. The general type of machinery in each case is the same-genetic material, the enzyme-forming systems, the enzymes required for the synthesis of these, and finally the end products, that is specific enzymes, proteins and the like. The machinery for cell division differs from that for specialized function only in the type of information present in the genes, transferred to the enzyme-forming systems, and appearing in the products produced. The machinery for cell division is complex only because it must reproduce all the essential parts of the cell. In comparison, the machinery for specialized function such as that required for specific syntheses, degradation, transport, conversion of chemical to physical energy, and the like is relatively simple, particularly if the specialized function depends upon the presence of a specific molecule like hemoglobin. Degrees of differentiation in a cell line, for example, may then be viewed in terms of the balance between the machinery for cell division and that for cell function. In the process of organ differentiation in embryonic development, the division machinery is maintained; in the adult it is lost only in a few systems such as those in nerve and striated muscle. In specific cell lines such as the erythrocyte the process of differentiation results in a complete loss of the division machinery in the end product, the completely differentiated cell. It should be noted that in mammalian cells the process of differentiation is associated with cell division. It is possible that cell division allows deletion of already formed ribonucleoprotein granules or enzyme-forming systems which, if not renewed because of non-functional genetic material, decrease in number by half in each daughter cell. This may be the mechanism which shunts the building blocks from one enzyme-forming system into another and allows one to predominate eventually.*

In a more practical term, how is it possible

to describe differentiation? Both cytoplasmic and nuclear differentiation can be analyzed in morphological, functional and chemical terms. Cytoplasmic differentiation is a familiar concept due to the great morphological differences which are obvious with the light microscope. The electron microscope has revealed additional alterations in the fine structure of cells [45]. These morphological differences derive from chemical differences which have been analyzed by a variety of methods. Variations in metabolic pathways have been described by isotopic methods [46]. The enzymatic components which must be responsible for many metabolic alterations have been examined extensively, and it has been postulated that a specific tissue could eventually be distinguished from another tissue by knowledge of the levels of certain enzymes. This form of analysis would yield an enzymatic profile specific for each tissue [47]. In addition to the identification of specific enzymes and proteins in the cytoplasm, some methods exist for the identification of particulate structures. For example, mitochondrial counts per cell have been performed [48], and by ultracentrifugation technics it has been possible to estimate the amount of material in the mitochondrial and microsomal fractions. No method is as yet available for identification of the different families of enzyme-forming systems or ribonucleoprotein granules present in the cytoplasm.

In contrast to the well established methods available for the description of cytoplasmic differentiation, evidence for nuclear differentiation has been procured only recently. The most striking experiments which demonstrate functional alteration in the nucleus are those of Briggs and King [49], who transplanted nuclei from cells of frog embryos in varying stages of development back into enucleated frog eggs. Nuclei from the blastula stage allowed normal development, but those from the endoderm of a late gastrula stage resulted in arrested development with deficiencies of ectoderm and mesoderm. These deficiencies could now be transferred by transplantation of blastula nuclei from one generation to the next, and the changes in the nuclei were therefore considered to be irreversible.

Besides this evidence of functional differentiation there is morphological evidence for chromosomal differentiation. Swellings which are known as "puffs" appear and disappear on

^{*} Evidence has recently been presented which suggests that in some bacterial systems the RNA template may actually be unstable and may require renewal through the presence of DNA [206].

giant chromosomes at specific loci in specific cells at specific times of development [50]. "Puffing," the formation of "Balbiani rings" (a process similar to puffing but occurring over more extensive sections of chromosomes) and the alterations noted in the lampbrush chromosomes of certain oocytes [51] are all thought to be reflections of differential chromosomal function.

There is also chemical evidence which supports the concept of nuclear differentiation. Chemical analyses of chromosomal material have revealed varying concentrations of RNA and protein in the nuclei of different cell lines. Also, varying concentrations of RNA have been noted by cytochemical and isotope technics in loops of the lampbrush chromosome [51] and experiments with H3-cytidine have shown an uptake into the RNA in puff regions of giant chromosomes which is greater than the incorporation into RNA in other sections of the chromosomes [33]. These experiments all point to differential chemical activity in chromosomal material and support the concept of nuclear differentiation. The constancy of the amount of DNA in different tissues of the same animal has been a strong argument for the identity of the genetic material in all cells [3]. However, the chromatographic separation of DNA isolated from different tissues of the same species reveals different patterns [9]. This suggests that the chemical nature of the DNA may be different in different tissues. Thus various lines of evidence support the concept of nuclear differentiation, which may eventually be shown to represent an alteration in the transfer of specific information in the form of synthesized ribonucleoprotein granules to the cytoplasm. This hypothesis remains to be tested.

The phenomenon of induction [53,54] is considered to be a major mechanism of differentiation and is unrelated to transduction or transformation. In brief, when one tissue comes into contact with another, the latter may undergo differentiation to become a distinct third type of tissue. This induction phenomenon involves transfer of material from the first tissue to the second. Induction can occur only at a certain time in the development of the tissues. The type of induction is rather specific and depends upon the inducing and induced tissues. As yet there is no good agreement as to the nature of the inducing material. In one system a protein with induction activity has

been purified [55]; in another, nucleic acids have been implicated [56]. In a manner somewhat similar to the process of induction, such low molecular weight compounds as vitamin A have been shown to affect differentiation of epithelial cells in tissue culture [57]. Thus an alteration of the chemical environment of the cell due to adjacent cells or to the addition of material in an in vitro system results in differentiation. The biological and biochemical distinction between differentiation, generally considered to be an irreversible process, and "modulation," a reversible process which is noted in tissue cultures when the morphology varies with alterations of the chemical environment, is not entirely clear.

These phenomena describe an alteration, but do not give any insight into the manner by which the alteration occurs. Several mechanisms have been described whereby chemical compounds in the environment of the cell can alter enzyme levels or function within the cell [58-60], and it is probable that these mechanisms play a part in differentiation. Adaptive enzyme formation (occasionally called induced enzyme formation but to be distinguished from the induction phenomenon) has been studied extensively in bacteria and has also been noted in animal tissues in in vivo and in vitro studies [61,62]. In this case the presence of the substrate for an enzyme stimulates the synthesis of more of that enzyme by the cell. The opposite phenomenon also occurs and is known as enzyme repression or feedback repression [63]. In this case high levels of the product of a chain of reactions will inhibit the synthesis of one or more of the enzymes in the sequence. For example, high levels of arginine inhibit the synthesis of the enzyme ornithine transcarbamylase which catalyzes the reaction between ornithine and carbamyl phosphate to form citrulline [63]. In pyrimidine biosynthesis the end products have been shown to inhibit the formation of the enzyme catalyzing the first reaction in the biosynthetic chain [64]. Repression, then, is a feedback mechanism whereby the products inhibit their own synthesis by inhibiting the synthesis of an enzyme. It is not known at present whether the mechanism of feedback repression occurs at the level of the enzyme-forming system, where it could affect the synthesis or release of the protein, or at the stage of synthesis of the enzyme-forming system [65]. There is another form of negative feedback control known as

feedback inhibition. In this case the product of a series of biosynthetic reactions inhibits one of the earlier steps in the sequence by competing with the substrate for the active site on the enzyme. For example, cytidylic acid inhibits its own synthesis by inhibiting one of the first steps in pyrimidine biosynthesis prior to the ring formation [66]. Thus negative feedback inhibition, in which the activity of an enzyme is decreased due to competitive inhibition, differs from negative feedback repression, in which the product actually inhibits the formation of the enzyme. These are known mechanisms by which low molecular weight compounds can affect the levels or activities of enzymes. Part of the induction phenomenon in differentiation may be explained in these terms, but it is unlikely that the primary event, due probably to high molecular weight materials such as proteins, occurs via these mechanisms.

In summary then, the process of differentiation involves both the nucleus and the cytoplasm, and eventually may be analyzed in terms of functional genes, enzyme-forming systems and end products. The machinery for cell reduplication is similar but obviously far more complex than that required for specialized function. It seems likely that differentiation involves a shifting of building blocks from one set of pathways into others, and the phenomenon of induction describes how the products of one tissue can bring about this shift in a second tissue. It is not known whether these alterations of molecular populations occur primarily through some type of enzyme adaptation or repression, or whether the primary alteration is on functional genetic material and this is then reflected by a change in enzyme synthesis. Answers to these problems will not be available until the mechanisms of information transfer between DNA and RNA are elucidated.

The Control of Cell Division. Knowledge concerning the precise mechanisms of cell division is extremely scanty despite the large number of experimental observations which have been made. Much of this evidence has been reviewed by Swann [67]. He has emphasized the fact that some of the division machinery, and in particular the spindle protein, must be synthesized prior to division. This low molecular weight protein, containing sulfhydryl or —SH groups capable of being oxidized to —S—S—intermolecular bonds [68], is estimated to occupy a relative volume of 10 to 30 per cent of some

rapidly dividing somatic cells [67]. Swann proposes that a balance exists between the synthesis of subcellular units for differentiation and the synthesis of those for cell division. These pathways may be mutually exclusive to some extent. The stimulus for cell division may be considered similar to the stimulus for cell differentiation, that is an induction phenomenon.

Many stimuli for cell division are known. Hormones represent the most obvious class of compounds, and of the animal hormones estrogen has received the most attention. Despite detailed data on the sequence of biochemical events in the uterus induced by the administration of hormones prior to cell division [69], and despite knowledge concerning the action of estrogen in a transhydrogenase reaction in which TPNH can reduce DPN in the presence of an enzyme and an estrogen derivative [70], it is not yet possible to pinpoint the exact mechanism by which cell division is triggered. The possibility that available TPN may stimulate the oxidation of glucose 6-phosphate to ribose 5-phosphate and thereby provide one of the building blocks for nucleic acid has no substantial proof. The plant hormones, the auxins and kinins, which affect cell size and division, are chemically defined compounds but their chemical action is not understood [71].

Mechanisms by which cell division in the animal is regulated through circulating substances not as yet classified as hormones have been described. The relationship of erythropoetin to red blood cell production is a poorly understood example of a control mechanism in which a decrease in a cell population may result in an increase of a stimulatory substance [72]. The control of growth in the regenerating liver after partial hepatectomy represents an example of a feedback mechanism. There is evidence now that the liver produces a substance which appears in the albumin fraction and which inhibits division of liver cells [73]. The experimental facts support the concept that when a portion of the liver is removed, the production of this material by liver cells is decreased, the inhibition of mitosis is thus relieved, and cell division occurs until the cell population is large enough to produce again adequate levels of inhibitor. This concept is similar to a general theory proposed by Weiss [74] which stated that a tissue controls its own growth rate by the production of inhibitory substances.

From this brief discussion, it is obvious that

there are a number of experimental systems in which the rate of cell division can be altered, in some cases by chemically defined materials. However, in any one of these systems there is little fundamental knowledge which pinpoints the actual chemical mechanism by which the compound affects the rate of cell division. This hiatus of understanding is primarily due to the lack of a comprehensive picture of the chemical events involved in cell division. With increasing knowledge of specific biosynthetic mechanisms at the enzymatic level, it will be possible eventually to develop an integrated picture of replication and its control at the cellular level.

General Aspects of Carcinogenesis. Burnet [75] has characterized cancer as "a process as inevitable as evolutionary progress and of the same general quality." This process he considers as a mutational event—an "inheritable change affecting cells at random and giving rise to a mutant clone (a family of cells) whose members can be shown to differ from those derived from unmodified individuals of the original population." Cancer as a biological process cannot be equated with evolution since the process itself results in the death of the host. Because the alteration occurs in somatic cells rather than in germinal cells, there are no possibilities for evolutionary alterations of the progeny of the organism. However, this does not deny that a common mechanism exists for evolution and

Statistical data concerning mortality rates have been used in the interpretation of the mutational basis of cancer. The death rate from cancer in man rises exponentially in proportion to approximately the sixth power of the age [76]. This mathematical analysis has been interpreted by Armitage and Doll [77] as indicating that there may be six mutational events responsible for the cancer if the mutant cells do not have a self-selective capacity, or more likely that a two-stage mutational process is required to produce a cancer cell provided the mutations give the resulting cells an advantage of self-selection over the normal population.

Intimately involved in the concept of mutation as a cause of cancer is the problem of genetic predisposition to cancer [78]. This is demonstrated readily through inbred strains of animals in which a high or low incidence for a specific form of tumor may be noted. An unusual aspect of this form of genetic control in animals is illustrated by the high cancer strain hybrids

which result from the crossing of some specific strains with low cancer incidence [78]. Genetic predisposition to cancer may be considered mechanistically in two ways. First, it is possible that there is an inherited structural alteration in genetic material so that a gene in a specific cell line of one strain of animal is more prone to mutation than is the corresponding gene in another strain. This would be comparable to the demonstration of mutational "hot-spots" in genes in transduction experiments [16]—areas of genetic material in which a high rate of mutation occurs compared to other areas. The second explanation for genetic predisposition to cancer involves the inherited alteration of enzymatic systems which are involved, for example, in the inactivation of endogenous or exogenous carcinogens. This is an indirect rather than a direct effect of the alteration of genetic material.

At the level of the organism, therefore, cancer can be considered in many instances as a mutational event different from the process of evolution only in that it occurs in somatic cells rather than in germ cells [79]. The relationship of mortality rate due to cancer with age and the genetic predisposition to cancer can be considered in this framework.

It is at the cellular and subcellular level rather than at the level of the whole organism that the problem of the nature of cancer must be explored. At this level, cancer is expressed in the biological property of uncontrolled cell division which is an hereditary characteristic of the cell line. This positive statement concerning the nature of cancer provides a basis for the construction of any hypothesis to explain in more detailed biochemical terms the nature of cancer. It is from the premise that this biological property represents an hereditary alteration that the importance of a consideration of the nucleic acids arises. Obviously, there is some alteration in information either possessed or expressed by the cancer cell and this alteration must involve the two information centers-DNA or chromosomal material and RNA or enzyme-forming systems [80]. It is possible to examine the former in a gross fashion, and a discussion of this will follow. Technics are not available by which any meaningful analysis of the enzyme-forming systems can be obtained. The question of what the alteration is which permits uncontrolled growth can be investigated by examining the end products of the DNA-RNA sequence. An analysis

of the morphology and enzymatic characteristics of the cancer cell will also be presented.

In an examination of the cancer cell for alterations in the information transfer system the first question to be answered is whether there are any criteria for alterations. From a functional viewpoint we have already defined the cancer cell in these terms. The fact that the alteration which allows uncontrolled growth is hereditary does not in any way localize the defect. In a consideration of what may appear to be hereditary features, it is worth emphasizing that an alteration of information transfer can be considered not only in terms of DNA and genes, but also in terms of self-duplicating RNA units which are independent of DNA. Some of the tumor viruses which are in the latter category will be discussed. For the present, however, alterations of information transfer will be considered in terms of chromosomal material.

Both quantitative and qualitative morphological changes have been noted in chromosomes of tumor cells. An alteration of the number of chromosomes is extremely common in tumors [81,82]. Polyploidy, a doubling, quadrupling, of the normal number, or aneuploidy, a chromosomal number which is not an integral of the diploid number, is found in most tumors. Indeed, one author [83] takes the extreme position that there is no good evidence in any tumor for the normal diploid number of chromosomes. The concept of genetic imbalance as a form of mutation has been stressed [78] and this implies a quantitative rather than a qualitative alteration in genes. Polyploidy or aneuploidy is frequently seen in normal cells when they are grown in tissue culture, and cells which morphologically resemble tumor cells occasionally appear. The question whether the alteration in chromosome number is the cause of the transformation to neoplastic-like cells or is merely associated with it has not been answered [83,84]. Alterations in the morphology of specific chromosomes have been noted in tumor cells which have not been seen in normal cells [85]. There is, however, no proof that these quantitative and qualitative morphological alterations are a sine qua non of the cancer cell. It seems likely that an alteration in the base sequence of a small portion of a DNA molecule would not necessarily be reflected in gross morphological changes. However, the imperfect understanding of chromosomal structure at a molecular level [86,87] makes predictions impossible.

At a chemical level there are as yet no alterations in DNA structure which are consistent for all tumors. The tools for analysis are too gross to describe the changes which might be significant.

Since it is not possible to demonstrate any specific defect in the genetic material per se. it is worth examining the cell itself to see if there are any unique alterations which would be a reflection of an alteration in the information transfer systems. An examination of the morphology of the cell might be expected to reveal some unique aspects. The light microscope has disclosed changes in the nuclear cytoplasmic ratio, nuclear chromatin, number and size of nucleoli, and alteration in the cytoplasm which, when considered together, are characteristic of cancer cells [88]. Some of the nuclear changes may be related to the increased number of chromosomes already noted. There is not anything unique about the nucleoli or the cytoplasmic changes. The electron microscope has not yet revealed any changes which are pathognomonic of tumor cells [89,90]. There may be some decrease in the membranous structures of the endoplasmic reticulum, with the appearance of more ribonucleoprotein granules free in the cytoplasm, but this is a quantitative alteration which is less obvious in some hepatomas for example [91] than in ascites cells [92]. Thus morphological analysis of tumor cells does not reveal any unique aspect, but only a general pattern to which most malignant cells conform.

Enzymatic analysis also has revealed a general pattern present in tumor cells which differs in many instances from that of the parent normal tissue. Greenstein [49,93] has presented a mass of impressive data to substantiate several conclusions he has reached. Each normal tissue has a characteristic pattern of cellular constitutents and levels of enzyme activity. Tumor tissues arising from these normal tissues have a much more narrow range of enzymatic activities, and the levels of these enzyme activities are independent of age, growth rate, or source of tumor. They seem to converge on a common pattern which is much like embryonic tissue. There is also a decrease or loss of many of the normal tissue's specific systems. Greenstein's hypothesis regarding the general enzymatic pattern of tumor tissue stems from Warburg's early observations on the rate of glycolysis of tumor tissues [94,95]. Warburg found that the rate of glycolysis, that is the conversion of glu-

cose to lactic acid, was greatly increased in tumor tissue as compared to normal tissues. He also considered that there was a decrease in respiration, that is the oxidation of substances in the Krebs cycle to carbon dioxide and water. He hypothesized that the initial injury which produced a cancer cell was to the respiratory system, to mitochondria which were capable of carrying out oxidation reactions and coupling the energy from these with the phosphorylation of ADP to form ATP. The cell, then a dormant cancer cell, could divide only slowly, but by the process of selection cells appeared with a high glycolytic capacity which could divide very rapidly. He believed that the shift from a dependence of energy derived mainly from respiration to energy derived in large part from glycolysis was directly responsible for the properties of dedifferentiation and of uncontrolled growth. Although the decrease in the respiratory rate of tumor cells is disputed [96,97], it is apparent that there are alterations in enzymatic levels of the energy-generating systems [98]. The question posed by these observations is whether some of the alterations in enzymatic capacities of the cell are primary and are the cause of the neoplastic process or whether they are secondary to the conditions imposed by rapid cell division due to some other cause. Warburg [94] has indicated that the primary event in the conversion of a normal to a neoplastic cell is the damage to respiration. It is unlikely that carcinogenic agents have a direct damaging effect upon mitochondria as he proposed, but it is not impossible that the defect may reside in some DNA-RNA system which ultimately affects the function of the respiratory systems of mitochondria. Evidence for the nucleic acids as primary targets in carcinogenesis will be presented in the following section. The question, however, of cause or effect cannot be answered at present.

From the evidence presented it is apparent that there are morphological and enzymatic alterations in the cancer cell which, when considered together, allow a general description of the cell. None of these changes, however, is unique for the cancer cell. There is much morphological and functional evidence which indicates that in the conversion from a normal cell to a neoplastic cell much of the machinery for specialized function is lost. This is evident for example in the decrease or loss of several enzymes from hepatomas [80]. This deletion of

specialized function in the transformation to the neoplastic cell is paralleled by a loss of organ-specific antigens [99]. For example, a chemically induced tumor of the liver loses the antigen which is specific for liver. More significant perhaps than this loss of antigenic material is the appearance of an antigen which may be specific for cancer tissue. This has been described by Bjorkland [100] and by Zilber [101] and is considered to be a lipoprotein which exists as a surface antigen. This represents the only qualitative alteration in cancer cells which has been described and may prove to be of great significance.

The presence of mutant cell lines within one tumor is well established. Morphologically there are differences apparent in some metastases compared to the primary tumor. Differences have been reported in drug sensitivity of strains of tumor cells arising from the same source [102], and differences in isoantigens also occur [103]. The probability that mutations are occurring continually in the cell population of a tumor, and that many of the mutations give the cells a selective advantage over the other cells, is the basis for the concept of progression of tumors

expressed by Foulds [104].

Considerable experimental evidence suggests that carcinogenesis by physical or chemical agents may represent a two-stage process. In the first stage some event occurs in the cell which at the time cannot be detected. In the second stage this event manifests itself after a gradual conversion of the affected cell to a cell with neoplastic properties. The mechanism of the primary event will be discussed under specific agents. This event may be an alteration in the nucleic acid structure of the cell. The second stage or conversion must represent in chemical terms a re-orientation of the enzymeforming systems of the cell and thus the end products within the cell. This alteration in molecular constituents requires time and may also depend upon cell division for the selection of the substrains which are best able to proliferate in the chemical environment. It is likely that this second stage is responsible for the variable latent periods noted with different carcinogenic stimuli.

In summary, cancer can be considered as due to a somatic mutation, in the broadest sense of the term. The relationship of mortality rate to age and the genetic predisposition to cancer can be interpreted in this light. At the cellular

level, the hereditary biological property of uncontrolled cell division characterizes cancer. An analysis of the genetic material present in cancer cells reveals quantitative and qualitative alterations from the normal. Analysis of the end products of functional genes reveals a morphological and enzymatic pattern which is characteristic of the cancer cell, but the only feature which so far has appeared as unique is the presence of a surface antigen not found in normal cells. In the critical area of mechanisms of control of cell division, no data are available, clearly because there is no well described mechanism which can be compared in normal and tumor tissue. This is the point upon which the analysis of an altered transfer of information founders, and it is precisely this point which must be clarified in order that the problems of control of cancer may be approached more rationally. The concept of carcinogenesis as a two-stage mechanism in which some irreversible alteration occurs in the cell and is followed by the gradual conversion of this altered cell into a cancer cell by mechanisms of adaptation was discussed. In the following section, an analysis of the mechanisms of carcinogenesis in terms of altered information transfer is outlined.

CARCINOGENESIS BY SPECIFIC AGENTS

Carcinogenic agents fall into three major categories: physical, chemical and viral. A brief consideration of each of these categories is warranted to indicate some of the biological aspects of carcinogenesis and furthermore to attempt to demonstrate in what manner specific carcinogens may affect subcellular organization and the programming of information within a cell.

Physical Agents as Carcinogens. The production of neoplasms of the epidermis by ultraviolet light has been observed in many species and the incidence and latent period has been studied as a function of the intensity and duration of the dose [105,106]. It has been concluded that the production of cancer by ultraviolet light is a continuous process from the first dose and that the process is cumulative [105]. The action spectrum for carcinogenesis is below 320 mu [106] while that for mutation in microorganisms has an optimum at 260 mµ, the point of maximum absorption of the nucleic acids [107]. Because of the action spectrum, as well as the inactivation of transforming DNA by ultraviolet light [108], the production of mutant

forms in bacteria has been considered to be due to an alteration of nucleic acid structure. There is similar suggestive but not definitive evidence that carcinogenesis by ultraviolet light may be due to an alteration in DNA.

Ionizing radiation in the form of x-rays, and electrons is well known to be carcinogenic, and tumors have been produced in a wide variety of species and in many tissues [109–111]. In such instances the latent period between radiation and appearance of the tumor is long in relation to the life span. For example, the peak incidence of leukemia after exposure to the Hiroshima and Nagasaki atomic bombs in 1946 occurred in 1951 [112]; the latent period in miners exposed to radon prior to development of carcinoma of the lung was approximately seventeen years [113]. The most plausible mechanism of action of ionizing radiation in the production of tumors seems to be related to its direct damage to and deletion of specific genetic material followed by gradual selection of cells with an advantage in replication. This assumption is based upon studies of the mechanism of action of ionizing radiation. Ionizing radiation will produce morphological abnormalities if it is directed towards the nucleus of a cell [111,114]. Following radiation a series of chromosomal abnormalities, breaks, stickiness and crosses, can be observed [115] as well as deletions of specific chromosomal segments which have been correlated with mutations [2]. Gross alterations in the DNA molecule following radiation have been described [116], as well as inhibition of DNA synthesis [117]. The inhibition of synthesis may be due either to a direct effect on DNA or to an effect on mitosis [118]. The production of a gene mutation by a single ionization has been postulated [119] and this has been substantiated in experiments on the inactivation of transforming DNA [108]. This type of evidence points to a direct effect of ionizing radiation on the DNA of the cell. It should be noted, however, that thymus tumors can develop in unradiated tissue transplanted into a radiated host [120]. Thus, although much data point toward an effect of ionizing radiation on DNA, there is no direct evidence that this is the mechanism of carcinogenesis. The linear relationship between activation of lysogenic strains of bacteriophage and the dose of radiation [121] suggests that other mechanisms could also be operative in carcinogenesis.

Chemical Agents as Carcinogens. The chemical

$$R-N$$
 CH_2-CH_2
 CH_2
 $CH_$

Fig. 4. Mechanism of action of nitrogen mustard on guanylic acid in DNA. ① Alkylation of 7-nitrogen, ② cleavage of glycosidic linkage, ③ hydrolysis of phosphodiester bond.

carcinogens may be divided conveniently into four categories: alkylating agents, polycyclic hydrocarbons and aromatic compounds containing nitrogen or sulfur, hormones and finally a group of miscellaneous compounds. Extensive reviews on these compounds have appeared [122–124]. It is the object of this discussion to consider primarily those aspects which relate to the effect of carcinogens on the transfer of information or more specifically to the DNA, RNA, protein triad.

The first category, the alkylating agents, includes such compounds as the nitrogen and sulfur mustards, triethylene melamine, the diepoxides, and compounds similar to Myleran® [122]. Because their biological action appears to be grossly similar to that of radiation, these compounds are termed radiomimetic. All these compounds possess a degree of chemical instability which allows them to form carbonium ions, and these ions are able to form covalent bonds with groups of high electron density such as amino, hydroxyl or phosphate groups. For optimum biological activity it is necessary that the compound possess at least two functional or alkylating groups [122].

Cytological evidence for the locus of action of these compounds implicates a nuclear mechanism. With high levels of drug, cell division can be inhibited while cell growth as manifest by an increase in cell size continues [125]. The primary action appears to be during the resting

stage of the mitotic cycle, but the resulting damage becomes manifest only during mitosis when chromosomal fragmentation, breaks, deficiencies and bridge formation occur. It is interesting to note that there is some predilection for specific points in short chromosomes [122].

Biological damage through drug action is evident in cell death. High levels of the compounds are toxic to most living systems and this forms the basis of their chemotherapeutic use. With regard to information transfer, Auerbach [126] observed that nitrogen mustard was a mutagenic agent in drosophila, thereby providing the first evidence that a chemical compound was capable of producing mutations. This has since been well documented in mice and in Escherichia coli. The action of the alkylating agents as mutagens is paralleled by their action as carcinogens in mice, rats and drosophila [127].

Chemical evidence suggests that DNA is the target of drug action. Nitrogen mustard reacts with DNA in vitro to decrease its viscosity, which is evidence for breaks in the rod-like structure of the molecules [128]. When an injection of nitrogen mustard is given to an animal and the DNA is then isolated and chromatographed, the elution profile is altered. The drug appears to produce a decrease in high molecular weight DNA which is normally present. This alteration of the normal pattern is similar to that observed when normal DNA is treated in vitro with nitrogen mustard and then chromatographed [9]. In a proposed mechanism of action of nitrogen mustard, the 7-nitrogen of the guanine moiety of DNA is alkylated preferentially [129] (Fig. 4, (1) and this produces a quaternary nitrogen and thus a positive charge in the hydrogenbonded area of the double strands of the DNA molecule. The glycosidic bond of guanylic acid ② with an alkyl group in the 7 position is labile at pH 7 at 37° [130], and it is possible that the degradation of DNA occurs because of this increased lability of the glycosidic linkage followed by hydrolysis of the phosphodiester linkage (3) [131]. A break in the polynucleotide chain should be evident in the replication process. The parallel mutagenic and carcinogenic activities of the alkylating agents suggest that these mechanisms may be the same.

In the second category of chemical carcinogens, which includes polycyclic hydrocarbons and aromatic compounds containing nitrogen or sulfur, there is no direct evidence that the

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mechanism of action is related to nucleic acids. However, there are some interesting observations which bear on this possibility. Considerable evidence has been presented by Berenblum [132] to support the hypothesis of a two-stage process in carcinogenesis. Initiation, the first stage, is produced by the carcinogen and represents an irreversible chemical alteration of the cell. Initiation produces a dormant tumor cell. Promotion, the second stage, involves the conversion of the dormant to an overt tumor cell by the application of a "co-carcinogen," a compound such as croton oil. The promoter or co-carcinogen does not produce a tumor when applied alone in comparable amounts. This experimental situation, which may actually be more complicated [133,134], suggests some irreversible alterations in the programing of information in the cell followed by a selection process, as already discussed. It is evident that many of these hydrocarbon carcinogens are mutagenic agents, but the correlation between mutagenic and carcinogenic activity is not necessarily close [127]. Data on the chemical mechanisms of action of these compounds are available only in limited amount. The exciting predictions of the theoretical organic chemists concerning the degree of carcinogenic activity of phenanthrene ring compounds, on the basis of the electron density at a specific region [135,136], have been fulfilled by the isolation, after the application of C14-dibenzanthracene to skin, of a derivative which was altered in this region [137]. This provided experimental evidence for the hypothesis that the active portion of the carcinogen in protein binding corresponded to the region of the molecule which had been predicted as the active portion. Evidence for protein binding of the azo dyes (butter yellow) which produce hepatomas in rats is more extensive [138]; furthermore, the disappearance of dye binding with the appearance of the tumor has been correlated with a decrease in a protein component with a certain electrophoretic mobility [139]. This has led to the hypothesis that the mechanism of action of the azo dye is through the binding and deletion of a protein. The functional significance of this protein component is unknown. It is difficult to see how continued synthesis of a protein could be stopped by the removal of this protein. Possibly the reason for the deletion of the protein is that binding occurs first in the microsome or ribonucleoprotein granule fraction [140], or

possibly in the nuclear fraction [138]. However, no further details are available which might allow a more sturdy hypothesis of the mechanism of action of these compounds to be constructed. Many of these compounds are weak mutagenic agents and whether their mode of action is in this area or through more complex

protein deletion is not clear.

Hormones, the third category of chemical carcinogens, cannot be easily related to problems of information transfer. Many of the experimental systems in which tumors arise can be interpreted in terms of an alteration of feedback control either of the secretion of a trophic hormone or of cell proliferation in such a system [141,142]. However, the question to be answered is whether the hormone is carcinogenic by virtue of its chemical structure or whether it results only in cell proliferation which in turn allows the selection of dormant mutant cells which are already present. Evidence against the former is the fact that most of the hormoneinduced tumors arise in the tissues which are directly involved in the specific hormonal control mechanism. Further evidence indicates that the incidence of carcinoma of the breast induced by the administration of estrogen in mice is not strictly cumulative when the hormone is administered intermittently, but depends upon the duration of treatment [143]. This evidence supports a permissive action rather than a primarily carcinogenic action of the hormone.

The fourth category of chemical carcinogens includes a variety of unrelated compounds such as inorganic chemicals, i.e., arsenicals and chromate, plastics, carbon tetrachloride and tannic acid [122]. No biochemical mechanisms for the action of these compounds as carcinogens have been defined. Many of them, such as carbon tetrachloride and plastics, appear to stimulate division of specific cell series, and it may be this proliferation which leads to the selection of mutant forms as discussed in relation to hor-

monal carcinogenesis.

In summary, the chemical carcinogens provide some information regarding the mechanisms of carcinogenesis. Reasonable evidence is accumulating to support the concept that the alkylating agents act directly on the DNA molecule, and that their mutagenic and carcinogenic actions may be similar. There is no substantial evidence that the polycyclic hydrocarbons, which are able to produce some type of irreversible alteration in the cell in the process of initiation, act directly on a DNA-RNA mechanism. Protein delection by the azo dyes is not an entirely satisfactory explanation of their mechanism of action. Hormones probably act by increasing the opportunity for selection of mutant cells rather than as initiating agents. For a large group of miscellaneous agents there are no obvious mechanisms.

Viral Agents as Carcinogens. The appearance of tumors following the injection of subcellular particles is a well recognized phenomenon in many species [144-149]. There is now adequate morphological, biological and chemical evidence to allow recognition of these subcellular particles or filterable agents as viruses. The theoretical importance of viruses as carcinogens in this discussion is related to the fact that all viruses contain nucleic acids. Some viruses contain only DNA, others only RNA. One can see immediately that virus infection could allow the introduction of information into the cell in the form of the virus nucleic acid. In this consideration of the possible mechanisms of conversion of a normal cell to a tumor cell attention will be focussed first on viruses containing DNA and then on viruses containing RNA.

There is reasonably good evidence that two of the virus-induced tumors contain DNA. The first of these is the polyoma virus [150], so-called because of its ability to produce multiple types of tumors. This virus was isolated from mouse lymphatic leukemia tissues and was carried in cultures of embryonic mouse tissues, and then given by injection to newborn mice. A remarkable variety of carcinomas and sarcomas appeared in different tissues [151], a phenomenon

that can now be reproduced readily.

The other virus known to contain DNA is that which produces papillomas in rabbits [152]. After scarification of the skin of a rabbit and application of the virus, papillomas appear in several weeks. Occasionally the papillomas progress to malignant carcinomas. In this system, it is of interest that the quantity of virus which can be isolated from tumors varies [153]. In the benign papillomas induced in one strain of rabbit the virus is easily recoverable while in another strain it is difficult to recover. In the carcinoma it is extremely difficult or impossible to demonstrate the virus even though transplantation of the carcinoma cells to recipients results in the appearance of specific antibodies to the virus. These observations led to the concept of "masking of the virus" in the tumor-the

existence of the virus in another form. A comparable phenomenon occurs with some bacterial viruses. "Masking" may, however, simply reflect a low titer of virus not detected by present assay procedures [153].

Concepts developed in microbial genetics and virology have been very helpful in considering mechanisms of carcinogenesis by viruses [154]. At the outset, however, it must be stated that analogies between animal tumor viruses and bacteriophage should be limited to animal viruses which contain DNA, since the bacterial viruses contain only DNA. The two concepts which have been most important are those of transduction [8] and lysogeny [155]. There are multiple examples of alteration of hereditary characteristics through the phenomenon of transduction, the transfer by a virus of genetic material of DNA from one bacterium to another. To demonstrate this transfer it is necessary that the infection with bacteriophage does not lyse the cell. The term lysogeny [155] describes the existence of a bacterial virus within a microorganism as a "provirus" which does not cause lysis. If the cell is lysed by mechanical means, it is not possible to find infective virus particles: However, the provirus can be activated in the cell by such agents as ultraviolet light, x-ray or chemical carcinogens to form virus progeny which then lyse the cell. It is this well established concept of lysogeny which provides some analogy for the concept of masking.

That a bacterial virus containing DNA can introduce information into a cell has thus been demonstrated in transduction experiments [8]. Also, it has been possible to measure enzymes immediately after infection of the cell by bacteriophage which eventually lyse the cell. Kornberg [156] and Greenberg [157] and their collaborators have shown the appearance of some new enzymes and alterations in the levels of others several minutes after infection. It is therefore apparent that in microbial systems several phenomena have been described in which new information is introduced into the cell by infection with a DNA-containing virus. Whether the mechanism of action of the polyoma virus and the rabbit papillomas virus is similar is at present only speculation.

RNA has been either identified or implicated in several of the animal tumor viruses. Reasonably good evidence exists that one of the fowl leucosis viruses [147] and the Rous sarcoma virus [158] contain RNA. There are no micro-

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bial systems comparable to bacteriophage from which one can draw analogies regarding the mechanism of action of RNA tumor viruses. It is evident that the virus propagates after infection of the cell and that in the Rous system it is passed on to daughter cells during cell division rather than by reinfection [159]. The myeloblastosis virus can be demonstrated by electron microscopy in the cytoplasm of marrow cells in which it is distinct from the endoplasmic reticulum ribonucleoprotein granules because of its 1,200 Å diameter and its dense central core surrounded by an outer membrane [160]. The simplest hypothesis would be that the RNA viruses are self-duplicating cytoplasmic units similar to the tobacco mosaic virus, which are able to alter metabolic pathways of the cell possibly through the production of an enzyme or a series of enzymes. It is perhaps significant that an ATP-ase has been demonstrated to be associated with the myeloblastosis virus [147]. In an alternative mechanism, the RNA virus in some manner could affect directly some of the DNA of the cell.

There are a series of biological phenomena due to tumor viruses which eventually must be explained in chemical terms. The time of appearance of tumors after injection of the virus agent is variable. Following injection of the virus causing erythroblastosis, death of the chicken occurs in nine days [144]. The speed with which the Rous agent produces morphological alteration of cells is remarkable [161]. Within eighteen to forty-eight hours after injection the fibroblasts develop transitional forms, and gross tumors appear in one to two weeks. In contrast to this is the six to twenty month latent period required for the development of mammary carcinomas in mice following injection of the agent at birth [162]. The rapid appearance of tumors due to the fowl viruses suggests that a large number of cells may be infected and converted initially to tumor cells. The conversion is rapid and reflects an immediate alteration of one or several enzyme systems. This can be contrasted to the carcinogenic process due to x-radiation or chemical agents in which there is probably an irreversible alteration of only one or several cells and then a gradual selection from these of cells with an ability to proliferate rapidly. The long latent period in some virus systems may not be due to the small number of cells parasitized, but rather to the presence of tumor-inducing information in only a small percentage of the virus

particles, or to conditions in the host, such as hormonal levels, which are not favorable at the time of infection [163].

The type of cell which can be infected and which can respond with a neoplastic alteration is of great interest. A tumor virus in a very young animal may produce only hemorrhagic phenomena [144]. The fowl leucosis virus in small quantities can be passed from bird to bird without causing tumors [164]. The mouse mammary tumor agent is found in many tissues after infection, but tumors appear only in one tissue and then only on hormonal stimulation [144]. For some virus tumor systems there appears to be a very rigid specificity regarding the tissue in which a tumor can be produced. Other viruses may be able to produce tumors in several tissues. A possible example of this is the polyoma virus [151] with its ability to produce a series of tumors. A similar phenomenon has been noted in avian erythroblastic leukemia in which sarcomas and carcinomas of the kidney are noted occasionally [144], and in a mouse lymphatic leukemia in which parotid gland tumors and sarcomas also are seen [165]. It will be necessary to prove that only one virus causes several types of tumors, and the evidence for or against this is not clear [166].

The possibility exists that a virus may introduce into a cell more information than that required for tumor growth. The avian sarcomas comprise a large complex of tumors. From eighteen different tumors of mesodermal origin, filterable agents have been isolated which on reinjection cause the same kind of tumor [167]. The most striking example was noted when material isolated from an osteochondrosarcoma was injected into normal tissue distant from bone. A sarcoma developed from the mesodermal elements which also contained the elements typical of an osteochondrosarcoma. Thus the virus was considered to bear not only growth promoting information, but also information regarding specific normal subcellular systems. Whether or not a pure preparation would do this is unknown. The question remains open whether the virus can introduce new information regarding synthesis of bone and cartilage or whether it activates latent systems already present in the cell. This has some bearing also on the broader question of the interrelationship between the agents causing the different fowl leucoses-lymphomatosis, erythroblastosis, and myeloblastosis and the avian sarcomas. Antiserum to one virus will neutralize some others [168] and one author [168] has speculated that since the lymphomatosis virus is the only agent which has been demonstrated to be contagious, it may be the transmissible stem virus while mutant forms of this virus produce the leukemias.

The production of neoplasms by the injection of nucleic acids represents one of the most exciting phases of research in viral tumors. That virus nucleic acid, free of protein, was infectious was demonstrated first with tobacco mosaic RNA [169,28] and since then with nucleic acid isolated from a series of non-tumor viruses [170]. Now there is good evidence that a nucleic acid, sensitive to DNA-ase but insensitive to RNA-ase, can be extracted from the polyoma virus and can cause tumors in mice [150]. The production of solid tumors in mice by a nucleic acid, possibly DNA, also has been reported [171]. A nucleic acid, presumably RNA, isolated from human leukemic tissue was shown to produce tumors in mice [172]. Other workers [173-175] have also reported tumors following the injection of nucleic acid or nucleoprotein. The work with infectious nucleic acid will require a great deal of evaluation, particularly in the light of the production of tumors in mice with herring sperm DNA [176]. However, there is a growing body of evidence which suggests that nucleic acid preparations can induce neoplasms. This represents the most direct evidence that the production of neoplastic changes involves nucleic acids and that this mechanism must be related to the problem of information transfer within the cell. The general concept of the introduction of a nucleic acid molecule into a cell which can then alter the metabolic pathways so as to transform the normal into a neoplastic cell does not describe a precise mechanism. This description must await further information of the mechanism of growth control.

In summary, there are model systems in animals in which tumors can be produced with viruses containing either DNA or RNA. In each case introduction of the nucleic acid may be considered to represent the introduction of new information. The mechanism of action of a tumor virus containing DNA has some counterpart in bacterial viruses which contain DNA, and the bacterial phenomena of transduction and lysogeny may have some relationship to the animal tumor systems. Several tumor viruses contain RNA. It seems most likely that these viruses are self-duplicating and that they are

able to affect cell metabolism, possibly by introducing an altering enzyme in a manner similar to the bacteriophage system. The speed with which some tumors appear may be due to the infection and conversion of a large number of normal cells to malignant cells. The ability of viruses to produce tumors in different tissues seems to vary. There is evidence that some viruses may carry information related not only to cell division but also to the subcellular units which, for example, are responsible for the appearance of an osteochondroma. Evidence is gradually accumulating that it is possible to induce tumors by the injection of nucleic acid material isolated from tumors. This is of great theoretical importance with regard to the general concept that altered information transfer is responsible for the conversion of normal to neoplastic tissue.

CHEMICAL AGENTS FOR THERAPY

It is significant that most of the chemical agents which prevent tumor growth do so by affecting nucleotide or nucleic acid metabolism. The major portion of this discussion will be concerned with metabolite antagonists (antimetabolites), their loci of action, and reasons for their success and failure as chemotherapeutic agents [177–179]. Two other forms of chemotherapy, the synthesis of abnormal macromolecules and the degradation of macromolecules, also will be discussed. Finally, it is important to try to indicate bases for new

chemotherapeutic approaches.

The concept of metabolite antagonists evolved from the elucidation by Woods [180] in 1940 of the mechanism of action of the sulfa drugs. The demonstration that the sulfonamide drugs inhibited growth by competing with para-aminobenzoic acid, the normal substrate for the synthesis of folic acid, led to the synthesis of a variety of compounds structurally related to normal metabolites in the cell. Success in the chemotherapy of microorganisms has depended upon the presence of unique metabolic pathways required for their growth which are not operative in the host. The synthesis of folic acid in some bacteria which is inhibited by sulfa drugs, as well as the synthesis of specific cell wall material in some microorganisms which is affected by penicillin [181], are two examples. As yet, no such unique and qualitatively different pathway necessary for growth has been

demonstrated to be present in tumor cells and absent in the host tissue. Indeed, considering the origin of tumor cells, such a pathway would seem unlikely. On the basis of the concepts developed in this review, the alteration in the tumor cell is more likely to be the loss or quantitative alteration of some existing pathway. The only mechanism of carcinogenesis which might be an exception to this deletion concept is viral carcinogenesis in which new information may be introduced into the cell in the form of viral DNA or RNA.

Tumor chemotherapy by metabolic antagonists has therefore been dependent upon differences in the levels of reactants in tumor tissue as compared to host tissue. It is for this reason that the therapeutic index, or the difference in the level of drug which affects the tumor and that which is toxic to the host, is almost invariably so small. A brief examination of the Michaelis-Menten formulation of enzyme action [182] makes this more clear.

 $\begin{array}{c} \text{Substrate (S)} + \text{Enzyme (E)} \rightleftarrows \text{Complex (ES)} \rightarrow \\ \text{Product (P)} + \text{Enzyme (E)} \end{array}$

In the conversion of a substrate (S) to a product (P), an enzyme substrate complex (ES) is formed. A competitive inhibitor (I) or metabolite antagonist can also form a reversible complex with the enzyme (EI) and thereby decrease the amount of enzyme available for combination with the substrate. Both the substrate and the inhibitor have specific affinities for the enzyme, and therefore compete for the active site on the enzyme. Obviously it is theoretically possible to alter any one of the components in the reaction, and it is such an alteration in the tumor cell compared to the normal cell which makes a compound toxic to the tumor cell only. In the tumor cell the level of inhibitor (I) may be higher than in the normal cell because of a more effective transport mechanism of the drug into the tumor cell [183,184] or a decreased destruction of the inhibitor within the tumor cell [185]. Also, in the tumor cell the level of available substrate (S) may be lower, the amount of enzyme (E) may be less, or the enzyme may be altered in its affinity for substrate (S) or inhibitor (I). Finally, in tumor cells the product (P) may be required in higher amounts per unit of time than in normal cells, or in normal tissue the product may be synthesized via another pathway. All of these alterations would result in a positive differential effect of an inhibitor on tumor tissues as compared to normal tissue. Incidentally, drug resistance can be visualized in similar terms [186–188], and the ability of an agent to suppress a tumor depends upon the absence of drug resistant lines of cells in the population.

Empirically it has been established that several metabolite antagonists are effective against certain tumors in animals and man. These include analogues of folic acid, purine analogues, and several compounds isolated from fermenta-

tion media of microorganisms.

The analogues of folic acid inhibit the conversion of folic acid, which is itself inactive as a cofactor, to the active form, tetrahydrofolic acid [189]. A deficiency of the active cofactor which is involved in one-carbon metabolism affects a series of reactions but to varying degrees. The most sensitive reaction may be the conversion of deoxyuridine to thymidine [190], which may occur at the nucleotide level and could be rate limiting in the synthesis of DNA; this is suggested by studies of microbial and animal cells [191,192]. It is obvious that the analogues of folic acid are designed to produce a folic acid deficiency. Whether this folic acid deficiency affects the growth rate of a tumor more than of normal cells then depends upon the degree of deficiency produced in each tissue and also upon which of the folic acid dependent enzymes is rate limiting in each system. This illustrates how crude the target is for which this compound was designed. Fortunately, in some of the acute leukemias of childhood [193] and in choriocarcinoma [194] these factors are so balanced that the folic acid analogue occasionally is effective.

A series of purine analogues have been synthesized, the most effective of which appears to be 6-mercaptopurine [195]. The mechanism of action of 6-mercaptopurine is still unresolved. It can be converted enzymatically to the ribotide [196] and mammalian cells resistant to 6-mercaptopurine are unable to do this [197]. Evidence has been presented which indicates inhibition of the conversion of inosinic acid to adenylic acid, inhibition of synthesis of cofactors such as DPN and CoA, and effects upon glycolysis and respiration [178,179]. These data suggest that 6-mercaptopurine antagonizes synthesis of adenylic acid and thereby affects several reactions. Again it is clear that

the action of this compound is at the nucleotide level and that its chemotherapeutic target is not well defined.

Of the compounds isolated from microorganisms which have activity against tumors, azaserine and DON (6-diazo-5-oxo-L-norleucine) have been found to inhibit reactions in nucleotide biosynthesis. Enzymatic steps in which glutamine donates its amide nitrogen to form either a purine intermediate from formylglycinamide ribotide [198] or a cytidine nucleotide from a uridine nucleotide [199] are inhibited by these compounds. The activity of these agents may be due to the ability of tumor cells to concentrate them better than normal cells [184].

All these metabolite antagonists inhibit the growth of only a few types of tumors. They have no effect on the majority of tumors arising from epithelial cells. This is because the compounds in tumor cells lack an advantage over normal cells in one or more of the terms noted in the Michaelis-Menten formulation. It is obvious that creating a deficiency of one or more of the nucleotides in a tumor cell, which is essentially the mechanism of action of the present antimetabolites, is a crude chemical dissection at best.

A different concept of chemotherapy is developing in which the aim is to design chemical agents which will be built into macromolecules and thereby disrupt their function. Evidence is now accumulating that some purine and pyrimidine analogues can be incorporated into nucleic acids. Increased frequency of mutation has been noted when 5-bromouracil was incorporated into phage DNA [200], and a loss of infectivity was observed when 8-azaguanine was incorporated into tobacco mosaic virus RNA [200]. This mechanism of growth inhibition may be important in tumor chemotherapy [201], but again it is evident that the target is rather non-specific.

Several chemical agents act by degrading or disorganizing macromolecules. The therapeutic action of alkylating agents such as nitrogen mustards, Myleran and the epoxides is considered to be on DNA and was discussed under chemical carcinogens. There is no reason for believing that the therapeutic action differs from the carcinogenic action in anything other than the dose of drug and the amount of DNA alkylated. These agents are not metabolite antagonists. Although they act on division and

information machinery, it is evident as with the other compounds that their action is not selective. It is well known that they are relatively ineffective against epithelial cell tumors in doses which are toxic to some normal cell systems.

There is some evidence that colchicine derivatives, which have been used clinically, are able to disrupt the polymerization of the spindle protein [202]. This emphasizes the fact that rates of cell division can be altered through systems other than the nucleic acids. For this reason these compounds are of theoretical importance, but practically they are active only in a few types of tumors [203].

Chemotherapeutic agents in these three categories-metabolite antagonists, compounds which alter macromolecular structure, and compounds which degrade macromolecules-are all designed for relatively non-specific chemical targets. It is easy to state that mechanisms involving control of cell division represent a more specific target, but these remain chemically undefined. Any definition of these targets involves an answer to the biochemical nature of the cancer cell. Possibly, the target is a control mechanism deficient in the cancer cell but operative in normal cells and re-establishment of the mechanism requires replacement. Macromolecular structures may be involved directly in such control. The shunting of substrate from one system of genes to another occurs in differentiation, and it may be necessary to draw upon ideas from this field, the area of control of genetic function, to develop effective chemotherapeutic concepts. It therefore seems possible that a consideration of the interaction of macromolecules in information transfer and the examination of mechanisms of control of cell division might be more productive than the design of low molecular weight antimetabolites which block general reactions [204]. Until the requisite knowledge is available, active agents must be sought for by empirical means [205]. This is undoubtedly worthwhile because active agents frequently become tools for the elucidation of mechanisms. It is possible that a theory of drug action will emerge which is based not on the concept of metabolite antagonists, but perhaps on a concept related to replacement of compounds which control cell division or upon information regarding the control of active genetic units. These theories must await more definitive information about the control of cell division and the mechanisms of induction and gene function.

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SUMMARY

It may be of some advantage to review, within the context of the problem of programing of information in the cell, some of the concepts which have been elaborated. An outline of the basic mechanisms of information transfer was indicated in the first section. It was emphasized that the information which resides in specific genes or DNA molecules must be coded by sequences of nucleotide pairs. This information must determine the sequence of amino acids in a specific protein synthesized under the direction of that particular gene or DNA molecule. An altered amino acid sequence in the protein may some day be shown to be due to an altered nucleotide sequence in the DNA molecule. The steps which allow the transfer of information from the DNA molecule to the protein involve the synthesis of specific RNA molecules present in the ribonucleoprotein granules of the endoplasmic reticulum (or microsome fraction). This is the major enzyme (or protein) forming system, and it is probably on a specific RNA, which acts in some manner as a template, that the amino acids are aligned in a sequence unique for the particular protein. Following peptide bond formation, the protein or enzyme molecule is released. This represents a gross outline of the transfer of information within the cell.

The processes of differentiation and carcinogenesis may both be viewed as alterations of the information transferred. It was indicated that cell constituents can be divided roughly into machinery for specialized function and machinery for cell division. In general terms the units of machinery are the same, i.e., DNA, RNA, protein. It is the specific information which differs. In any line of cells undergoing differentiation, however, there is a changing emphasis between machinery for division and machinery for specialized function. In the extreme case of differentiation, as in the mature red blood cell, only machinery for specialized function is retained while all division machinery is lost. The opposite extreme is the anaplastic carcinoma cell in which division machinery is maintained with the loss of all obvious specialized function.

At the present time it is possible to analyze nuclear and cytoplasmic differentiation in morphological, functional and chemical terms. It is apparent that there exist nuclear-cytoplasmic mechanisms which remain undescribed in chemical terms and which are responsible for maintenance of increased function of some genetic units in a cell and loss of function of others. The phenomenon of induction noted in embryonic development describes differentiation in one cell line due to the transfer of material from an adjacent cell line. What this material is and how it acts is at present unknown. Whether this phenomenon operates through any of the known control mechanisms, such as enzyme inhibition, enzyme repression or enzyme adaptation, also cannot be answered.

The cancer cell possesses an hereditary defect in the normal mechanism of control of cell division. Since the nucleic acids form a chemical basis of heredity in the cell it is reasonable to suspect an alteration in nucleic acids in the process of carcinogenesis. This implies some sort of qualitative alteration in the information transfer system. (By qualitative is meant either a direct physicochemical effect which alters the structural configuration of a nucleic acid molecule, or a mechanism whereby foreign nucleic acid molecules are incorporated into the cell.) Evidence has been presented suggesting but not proving that some of the carcinogenic agents may affect the genetic material or DNA molecules. Included in this series are ultraviolet and x-radiation, chemicals such as the nitrogen mustards and possibly the polycyclic hydrocarbons, and the DNA-containing polyoma and papilloma viruses. Affecting information transfer at the RNA level are tumor viruses which contain RNA. The mechanism of their action is obscure, but it seems reasonable to place them in this category. Thus mechanistically there are several ways of producing the same biological phenomenon, the cancer cell. Assuming that this premise concerning altered information in the cancer cell is correct, the next problem involves the type of information which is altered. Here we can only describe the biological property of uncontrolled growth and state that the alteration leads to loss of some control mechanism. The question regarding the exact biochemical nature of the control mechanism remains unanswered.

A number of the mechanisms described in the previous sections may be classified grossly as quantitative effects, possibly because of a lack of understanding of their true nature. The induction phenomenon may be considered as a quantitative alteration in the transfer of information from genes to enzyme-forming systems

which involves the concept of functional genes. of morphological changes such as puffing and Balbiani rings, and chemical changes in chromosomal material. Enzyme adaptation and repression are control phenomena which occur either at the level of the functional genetic unit or at the level of the enzyme-forming system. Polyploidy and aneuploidy also may represent a quantitative change in the number of genetic units, although it is possible to envisage the complete loss of some units. Many of the changes noted in a cancer cell in terms of enzyme levels may be merely quantitative changes which occur as an adaptation to rapid cell division. Some of the changes in glycolysis, for example, noted during the process of promotion by chemical agents may represent steps of adaptation for rapid cell proliferation. Although this division of phenomena into qualitative and quantitative categories is an artificial and somewhat arbitrary way to consider all of these biological processes, it does serve temporarily at least to categorize them in relation to information transfer.

Carcinogenesis, when envisaged as a twostage process of initiation and promotion, may involve both types of changes described here as qualitative and quantitative. The process of initiation may involve some irreversible alteration in the information transfer system which in itself does not result in a recognizable cancer cell. Promotion may then be a series of quantitative changes which occur in the cell during its ensuing divisions, resulting in loss of specialized machinery and gain in machinery for cell division. The end result of both of these processes is the cancer cell.

The whole field of chemotherapy of cancer suffers from a lack of fundamental knowledge concerning the biochemical nature of the cancer cell. It is possible that with an increasing understanding of the chemical nature of differentiation and growth controlling mechanisms, it will be possible to develop an entirely new concept of chemotherapy, based not upon metabolic antagonists of relatively simple chemical structure, but either upon replacement of missing information or end products in the cell, or possibly upon mechanisms of diversion of synthetic machinery from one end product or series of end products to another.

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REFERENCES

- Morgan, T. H. The Physical Basis of Heredity. Philadelphia, 1919. J. B. Lippincott Co.
- Srb, A. M. and Owen, R. D. General Genetics. San Francisco, 1957. W. H. Freeman & Co.
- VENDRELY, R. The deoxyribonucleic acid content of the nucleus. In: Chargaff, E. and Davidson, J. N. The Nucleic Acids. vol. 2, p. 155. New York, 1955. Academic Press.
- Griffith, F. The significance of pneumococcal types. J. Hyg. C., 27: 113, 1928.
- AVERY, O. T., MAGLEOD, C. M. and McCARTY, M. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. J. Exper. Med., 79: 137, 1944.
- ZINDER, N. D. and LEDERBERG, J. Genetic exchange in salmonella. J. Bact., 64: 679, 1952.
- HOTCHKISS, R. D. The biological role of deoxypentose nucleic acid. In: Chargaff, E. and Davidson, J. N. The Nucleic Acids, vol. 2, p. 435. New York, 1955. Academic Press.
- 8. Hartman, P. E. Transduction: a comparative review. In: McElroy, W. D. and Glass, B. The Chemical Basis of Heredity, p. 408. Baltimore, 1957. The Johns Hopkins Press.
- BENDICH, A., PAHL, H. B. and BEISER, S. M. Chromatographic fractionation of deoxyribonucleic acids with special emphasis on the transforming factor of pneumococcus. *Cold Spring Harbor Symposia Quant. Biol.*, 21: 31, 1956.
- Watson, J. D. and Crick, F. H. C. Molecular structure of nucleic acids. *Nature*, *London*, 171: 737, 1953
- WATSON, J. D. and CRICK, F. H. C. Genetical implications of the structure of deoxyribonucleic acid. *Nature*, *London*, 171: 964, 1953.
- SHOOTER, K. V. The physical chemistry of deoxyribonucleic acid. Progr. Biophys. & Biphys. Chem., 8: 309, 1957.
- Meselson, M. and Stahl, F. W. The replication of DNA in E. coli. Proc. Nat. Acad. Sc., 44: 671, 1958.
- LEHMAN, I. R., ZIMMERMAN, S. B., ADLER, J. BESS-MAN, M. J., SIMMS, E. S. and KORNBERG, A. Enzymatic synthesis of deoxyribonucleic acid. v. Chemical composition of enzymatically synthesized deoxyribonucleic acid. *Proc. Nat. Acad.* Sc., 44: 1191, 1958.
- CRICK, F. H. C., GRIFFITH, J. S. and ORGEL, L. E. Codes without commas. Proc. Nat. Acad. Sc., 43: 416, 1957.
- BENZER, S. The elementary units of heredity. In: McElroy, W. D. and Glass, B. The Chemical Basis of Heredity, p. 70. Baltimore, 1957. Johns Hopkins Press.
- Ingram, V. M. Chemistry of the abnormal hemoglobins. Brit. M. Bull., 15: 27, 1959.
- Childs, B. Hereditary aspects of disease. Bull. New York Acad. Med., 35: 77, 1959.
- Bretthauer, R. K., Hansen, R. G., Donnell, G. and Bergren, W. R. A procedure for detecting carriers of galactosemia. *Proc. Nat. Acad. Sc.*, 45: 328, 1959.
- Brachet, J. Biochemical Cytology. New York, 1957. Academic Press.
- 21. STEPHENSON, M. L., HECHT, L. I., LITTLEFIELD,

AMERICAN JOURNAL OF MEDICINE

- J. W., LOTFIELD, R. B. and ZAMECNIK, P. C. In: HAYASHI, T. Subcellular Particles, p. 160, New York, 1959. Ronald Press.
- LOTFIELD, R. The biosynthesis of protein. Progr. Biophys. & Biophys. Chem., 8: 347, 1957.
- SIMKIN, J. L. Protein biosynthesis. Ann. Rev. Biochem., 28: 145, 1959.
- COHEN, G. N. and GROS, F. Protein biosynthesis. Ann. Rev. Biochem., 29: 525, 1960.
- ROBERTS, R. B. Microsomal Particles and Protein Synthesis. New York, 1958. Pergamon Press.
- PALADE, G. E. The endoplasmic reticulum. J. Biophys. & Biochem. Cytol., 2: 85, 1956.
- PALADE, G. E. and SIEKEVITZ, P. Liver microsomes.
 J. Biophys. & Biochem. Cytol., 2: 171, 1956.
- 28. FRAENKEL-CONRAT, H., SINGER, B. A. and WILLIAMS, R. C. The nature of the progeny of virue reconstituted from protein and nucleic acid of different strains of tobacco mosaic virus. In: McElroy, W. D. and Glass, B. The Chemical Basis of Heredity, p. 501. Baltimore, 1957. Johns Hopkins Press.
- MUNDRY, K. W. and GIERER, A. Die Erzeugung von Mutationen des Tabak-mosaik durche chemische Behandlung seiner Nucleinsäure in vitro. Zsehr. Vererbungslehre, 89: 614, 1958.
- Reddi, K. K. The arrangement of purine and pyrimidine nucleotides in tobacco mosaic virus nucleic acid. Proc. Nat. Acad. Sc., 45: 293, 1959.
- SMELLIE, R. M. S. The metabolism of the nucleic acids. In: Chargaff, E. and Davidson, J. N. The Nucleic Acids, vol. 2, p. 393. New York, 1955. Academic Press.
- Pelc, S. R. and Howard, A. Metabolic activity of salivary gland chromosomes in diptera. Exper. Cell. Res., 10: 549, 1956.
- RUDKIN, G. T. and Woods, P. S. Incorporation of H³ cytidine and H³ thymidine into giant chromosomes of drosophila during puff formation. *Proc. Nat. Acad. Sc.*, 45: 997, 1959.
- GOLDSTEIN, L. and PLAUT, W. Direct evidence for nuclear synthesis of cytoplasmic ribose nucleic acid. Proc. Nat. Acad. Sc., 41: 874, 1955.
- SANGER, F. Chemistry of insulin. Science, 129: 1340, 1959.
- HILL, R. L., KIMMEL, J. R. and SMITH, E. L. The structure of proteins. Am. Rev. Biochem., 28: 97, 1959.
- 37. Anfinsen, C. B. The Molecular Easis of Evolution. New York, 1959. John Wiley & Sons, Inc.
- McElroy, W. D. and Glass, B. The Chemical Basis of Development. Baltimore, 1958. Johns Hopkins Press.
- Genetic mechanisms. Structure and function. Cold Spring Harbor Symposia Quant. Biol., 21: 1956.
- Weiss, P. Some introductory remarks on the cellular basis of differentiation. J. Embryol. Exper. Morph., 1: 181, 1953.
- Needham, J. Developmental physiology. Ann. Rev. Physiol., 17: 37, 1955.
- EPHRUSSI, B. Enzymes in cellular differentiation. In: GAEBLER, O. H. Units of Biological Structure and Function, p. 29. New York, 1956. Academic Press.

- Sonneborn, T. M. The gene and cell differentiation. Proc. Nat. Acad. Sc., 46: 149, 1960.
- 44. Rusch, H. P. Carcinogenesis: A facet of living processes. *Cancer Res.*, 14: 407, 1954.
- FAWGETT, D. W. Changes in the fine structure of the cytoplasmic organelles during differentiation. In: RUDNICK, D. Developmental Cytology, p. 161. New York, 1959. Ronald Press.
- MARKERT, C. L. The ontogeny of divergent metabolic patterns in cells of identical genotype. Cold Spring Harbor Symposia Quant. Biol., 21: 339, 1956.
- Greenstein, J. P. Some biochemical characteristics of morphologically separable cancers. *Cancer Res.*, 16: 641, 1956.
- STRIEBICH, M. J., SHELTON, E. and SCHNEIDER, W. D. Quantitative morphological studies on the livers and liver homogenates of rats fed 2-methyl or 3-methyl-4-dimethylaminoazobenzene. Cancer Res., 13: 279, 1953.
- King, T. J. and Briggs, R. Serial transplantation of embryonic nuclei. Cold Spring Harbor Symposia Quant. Biol., 21: 27, 1956.
- Quant. Biol., 21: 27, 1956.
 50. BEERMANN, W. Nuclear differentiation and functional morphology of chromosomes. Cold Spring Harbor Symposia Quant. Biol., 21: 217, 1956.
- Gall, J. G. Chromosomal differentiation. In: Mc-Elroy, W. D. and Glass, B. The Chemical Basis of Development, p. 103. Baltimore, 1958. The Johns Hopkins Press.
- The relationship between nucleus and cytoplasm. Exper. Cell. Res. (supp. 6) 1959.
- GROBSTEIN, C. Differentiation of verebrate cells.
 In: BRACHET, J. and MIRSKY, A. E. The Cell,
 p. 437. New York, 1959. Academic Press.
- YAMADA, T. Embryonic induction. In: McElroy, W. D. and Glass, B. The Chemical Basis of Development, p. 217. Baltimore, 1958. Johns Hopkins Press.
- COHEN, S. Purification of a nerve growth promoting protein from the mouse salivary gland its neurocytotoxic antiserum. *Proc. Nat. Acad. Sc.*, 46: 302, 1960.
- 56. Niu, M. C. New approaches to the problem of embryonic induction. In: Rudnick, D. Cellular Mechanisms in Differentiation and Growth, p. 155. Princeton, 1956. Princeton University Press.
- 57. Weiss, P. and James, R. Skin metaplasia in vitro induced by brief exposure to vitamin A. Exper. Cell. Res., supp. 3, p. 318, 1955.
- PARDEE, A. B. The control of enzyme activity. In: BOYER, P., LARDY, H. and MYRBACK, K. The Enzymes, vol. 1, p. 681. New York, 1959. Academic Press.
- POTTER, V. R. and AUERBACH, V. H. Adaptive enzymes and feedback mechanisms. *Lab. Invest.*, 8: 495, 1959.
- POLLOCK, M. R. Induced formation of enzymes. In: BOYER, P. O., LARDY, H. and MYRBACK, K. The Enzymes, vol. 1, p. 619. New York, 1959. Academic Press.
- 61. KNOX, W. E., AUERBACH, V. H. and LIN, E. C. C. Enzymatic and metabolic adaptations in animals. *Physiol. Rev.*, 36: 164, 1956.
- 62. DeMars, R. The inhibition by glutamine of glutamyl transferase formation in cultures of

- human cells. Biochem. et biophys. acta, 27: 435, 1958.
- 63. Gorini, L. and Maas, W. Feedback control of the formation of biosynthetic enzymes. In: McElroy, W. D. and Glass, B. The Chemical Basis of Development, p. 469. Baltimore, 1958. Johns Hopkins Press.
- 64. YATES, R. A. and PARDEE, A. B. Control by uracil of formation of enzymes required for orotate synthesis. *J. Biol. Chem.*, 227: 677, 1957.
- 65. Vogel, H. J. Comment on the possible roles of repressers and inducers of enzyme formation in development. In: McElroy, W. D. and Glass, B. The Chemical Basis of Development, p. 479. Baltimore, 1958. Johns Hopkins Press.
- YATES, R. A. and PARDEE, A. B. Control of pyrimidine biosynthesis in *Escherichia coli* by a feedback mechanism. *J. Biol. Chem.*, 221: 757, 1956.
- SWANN, M. M. The control of cell division: a review. Cancer Res., 17: 727, 1957; 18: 1118, 1958.
- 68. MAZIA, D. Materials for the biophysical and biochemical study of cell division. Advances Biol. Med. & Physics, 4: 69, 1956.
- MUELLER, G. C. A discussion of the mechanism of action of steroid hormones. Cancer Res., 17: 490, 1957.
- HAGERMAN, D. D. and VILLEE, C. A. Separation of human placental estrogen sensitive transhydrogenase from estradiol-17β dehydrogenase. J. Biol. Chem., 234: 2031, 1959.
- Braun, A. C. A physiological study of the nature of autonomous growth in neoplastic plant cells. In: Symposia of the Society for Experimental Biology, vol. 11, p. 132. New York, 1957. Academic Press.
- JACOBSON, L. O., GOLDWASSER, E., GURNEY, C. W., FRIED, W. and PLZAK, L. Studies of erythropoetin: the hormone regulating red cell production. Ann. New York Acad. Sc., 77: 551, 1959.
- 73. GLINOS, A. D. The mechanism of liver growth and regeneration. In: McElroy, W. D. and Glass, B. The Chemical Basis of Development, p. 813. Baltimore, 1958. Johns Hopkins Press.
- Weiss, P. and Kavanau, J. L. A model of growth and growth control in mathematical terms. J. Gen. Physiol., 41: 1, 1957.
- 75. Burnet, F. M. Opening remarks, the biology of the cancer cell. Fed. Proc., 17: 687, 1958.
- ARMITAGE, P. and DOLL, R. The age distribution of cancer and a multistage theory of carcinogenesis. *Brit. J. Cancer*, 8: 1, 1954.
- Armitage, P. and Doll, R. A two-stage theory of carcinogenesis in relation to the age distribution of human cancer. *Brit. J. Cancer*, 11: 161, 1957
- 78. Huxley, J. Biological Aspects of Cancer. New York, 1958. Harcourt, Brace & Co.,
- BURNET, M. Cancer—a biological approach. *Brit. Med. J.*, 1: 779, 841, 1957.
- 80. POTTER, V. R. The biochemical approach to the cancer problem. *Fed. Proc.*, 17: 691, 1958.
- 81. Strong, L. C. Genetic concept for the origin of cancer: historical review. *Ann. New York Acad. Sc.*, 71: 810, 1958.
- 82. Klein, G. and Klein, E. Nuclear and cytoplasmic changes in tumors. In: Rudnick, D. Develop-

- mental Cytology, p. 63. New York, 1959. Ronald Press.
- Hsu, T. C. Numerical variation of chromosomes in higher animals. In: Rudnick, D. Developmental Cytology, p. 47, New York, 1959. Ronald Press.
- Levan, A. and Biesele, J. J. Role of chromosomes in carcinogenesis, as studies in serial tissue culture of mammalian cells. *Ann. New York Acad. Sc.*, 71: 1022, 1958.
- Makino, S. Further evidence favoring the stem cell in ascites tumors of rats. Ann. New York Acad. Sc., 63: 818, 1956.
- RIS, H. Chromosome structure. In: McElroy, W. D. and Glass, B. The Chemical Basis of Heredity, p. 23. Baltimore, 1957. Johns Hopkins Press.
- STEFFENSEN, D. A comparative view of the chromosome. In: Brookhaven Symposium in Biology, Structure and Function of Genetic Elements, 1959.
- 88. Florey, H. General Pathology, Philadelphia, 1958. W. B. Sanders.
- Bernhard, W. Electron microscopy of tumor cells and tumor viruses. Cancer Res., 18: 491, 1958.
- Dalton, A. J. and Felix, M. D. The electron microscopy of normal and malignant cells. Ann. New York Acad. Sc., 63: 1117, 1956.
- Dalton, A. J. Organization in benign and malignant cells. Lab. Invest., 8: 510, 1959.
- Selby, C. C., Biesele, J. J. and Grey, C. E. Electron microscope studies of ascites tumor cells. Ann. New York Acad. Sc., 63: 748, 1956.
- Greenstein, J. P. The Biochemistry of Cancer, 2nd ed. New York, 1954. Academic Press.
- WARBURG, O. On the origin of cancer cells. Science, 123: 309, 1956.
- WARBURG, O., BURK, O. and SCHADE, A. L. On respiratory impairment in cancer cells. Science, 124: 269, 1956.
- Weinhouse, S. Oxidative metabolism of neoplastic tissue. Advances Cancer Res., 3: 269, 1955.
- Weinhouse, S. On respiratory impairment in cancer cells. Science, 124: 267, 1956.
- POTTER, V. R. Biochemical uniformity and heterogeneity in cancer tissue. Cancer Res., 16: 658, 1956.
- WEILER, E. C. W. The loss of specific cell antigen in relation to carcinogenesis. In: Ciba Foundation Symposium on Carcinogenesis, p. 165. Boston, 1959. Little, Brown & Co.
- 100. BJORKLAND, B. and BJORKLAND, V. Antigenicity of pooled human malignant and normal tissues by cytoimmunological techniques: presence of an insoluble heat labile tumor antigen. *Internat. Arch. Allergy*, 10: 153, 1957.
- Vygodchikov, G. V. Pathogenesis and Immunology of Tumors. New York, 1959. Pergamon Press.
- Law, L. W. Some aspects of drug resistance in neoplasma. Ann. New York Acad. Sc., 71: 976, 1958.
- 103. Hauschka, T. S. Kvedar, B. J., Grinnell, S. T. and Amos, D. B. Immunoselection of polyploids from predominately diploid cell populations. Ann. New York Acad. Sc., 63: 683, 1956.
- FOULDS, L. Neoplastic development. In: McElroy,
 W. D. and Glass, B. The Chemical Basis of

Development, p. 680. Baltimore, 1958. Johns Hopkins Press.

- Велим, Н. I. Carcinogenesis by ultraviolet light: an essay in quantitative biology. Princeton, 1959. Princeton University Press.
- Blum, H. F. On the mechanism of cancer induction by ultraviolet radiation. J. Nat. Cancer Inst., 11: 463, 1950.
- HOLLAENDER, A. and Emmons, C. W. Wave length dependence of mutation production in the ultraviolet with special emphasis on fungi. Cold Spring Harbor Symposia Quant. Biol., 9: 179, 1941.
- LERMAN, L. S. and TOLMACH, L. J. Genetic transformation. Biochim. et biophys. acta, 33: 371, 1959.
- BRUES, A. Ionizing radiations and cancer. Advances Cancer Res., 2: 177, 1954.
- WARREN, S. Carcinogenesis by radiation. Cancer Res., 17: 1, 1957.
- UPTON, A. C. The radiobiology of the cancer cell. Fed. Proc., 17: 678, 1958.
- COURT-BROWN, W. M. Nuclear and allied radiations and the incidence of leukemia in man. Brit. M. Bull., 14: 168, 1958.
- 113. Kennaway, E. L. and Kennaway, N. M. The relation between the incidence and the incubation period of cancer in man. Yale, J. Biol. Med., 17: 139, 1944.
- Ciba Foundation Symposium, Ionizing Radiations and Cell Metabolism. London, 1956. Churchill.
- Puck, T. Action of radiation on mammalian cells. Proc. Nat. Acad. Sc., 44: 772, 1958.
- BUTLER, J. A. V. The action of ionizing radiations on biological materials, facts, and theories. *Radiat. Res.*, 4: 20, 1956.
- 117. ORD, M. G., STOCKEN, L. A., LAJTHA, L. G., OLIVER, R., BERRY, R. and NOYES W. D. Studies in synthesis of deoxyribonucleic acid. *Nature*. *London*, 182: 1787, 1958.
- 118. Kelley, L. The effects of radiation on DNA synthesis in mammalian cells. Progr. Biophys. & Biophys. Chem., 8: 143, 1957.
- Lea, D. E. Actions on Radiation on Living Cells. New York, 1947. Macmillan.
- KAPLAN, H. S., CARNES, W. H., BROWN, M. B. and HIRSCH, B. B. Indirect induction of lymphomas in irradiated mice. *Cancer Res.*, 16: 422, 1956.
- MARCOVICH, H. A quantitative biological test sensitive to low doses of ionizing radiations. Nature, London, 174: 796, 1954.
- 122. Haddow, A. Chemical and genetic mechanisms of carcinogenesis. In: Homberger, F. and Fishman, W. H. Physiopathology of Cancer, p. 441. New York, 1953. P. B. Hoeber.
- HADDOW, A. The biochemistry of cancer. Ann. Rev. Biochem., 24: 689, 1955.
- MILLER, E. C. and MILLER, J. A. Biochemistry of carcinogenesis. Ann. Rev. Biochem., 28: 291, 1959.
- PHILIPS, F. S. Recent contributions to the pharmacology of bis (2 haloethyl) amines and sulphides. *Pharmacol. Rev.*, 2: 281, 1950.
- AUERBACH, C. and ROBSON, J. M. Chemical production of mutations. *Nature*, *London*, 157: 302, 1946.
- 127. Burdette, W. J. The significance of mutation in relation to the origin of tumors: a review. Cancer Res., 15: 201, 1955.

- 128. Conway, B. E., Gilbert, L. and Butler, J. A. V. The action of ionizing radiations and of radiomimetic chemicals on deoxyribonucleic acid: III. The molecular weights of deoxyribonucleic acid degradation by x-rays and by treatment with a "nitrogen mustard." J. Chem. Soc., 3421, 1950.
- 129. LAWLEY, P. D. The relative reactivities of deoxyribonucleotides and of the bases of DNA towards alkylating agents. Biochim. et biophys. acta, 26: 450, 1957.
- LAWLEY, P. D. Hydrolysis of methylated deoxyguanylic acid at pH 7.0 to yield 7-methylguanine. Proc. Chem. Soc., p. 290, 1957.
- 131. BROWN, D. M. and TODD, A. R. Evidence on the nature of the chemical bonds in nucleic acids. In: CHARGAFF, E. and DAVIDSON, J. N. The Nucleic Acids, vol. 1, p. 409. New York, 1955. Academic Proces.
- Berenblum, I. Carcinogenesis and tumor pathogenesis. Advances Cancer Res., 2: 129, 1954.
- SALAMON, M. H. Cocarcinogenesis. Brit. M. Bull., 14: 116, 1958.
- 134. ORR, J. W. The mechanism of chemical carcinogenesis. *Brit. M. Bull.*, 14: 99, 1958.
- Pullman, A. and Pullman, B. Electronic structure and carcinogenic activity of aromatic molecules. Advances Cancer Res., 3: 117, 1955.
- 136. Coulson, C. A. Electronic configuration and carcinogenesis. *Advances Cancer Res.*, 1: 1, 1953.
- Heidelberger, C. Biochemistry of cancer. Ann. Rev. Biochem., 25: 573, 1956.
- MILLER, J. A. and MILLER, E. C. The carcinogenic aminoazo dyes. Advances Cancer Res., 1: 339, 1953.
- 139. SOROF, S., YOUNG, E. M. and OTT, M. G. Soluble liver proteins during hepatocarcinogenesis by aminoazo dyes and 2-acetylaminofluorene in the rat. Cancer Res., 18: 33, 1958.
- HULTIN, T. Reactions of C¹⁴ labeled carcinogenic azo dyes with rat liver proteins. Exper. Cell Res., 13: 47, 1957.
- 141. HERTZ, R. An appraisal of the concepts of endocrine influence on the etiology, pathogenesis and control of abnormal and neoplastic growth. Cancer Res., 17: 423, 1957.
- BIELSCHOWSKY, F. and HORNING, E. S. Aspects of endocrine carcinogenesis. *Brit. M. Bull.*, 14: 106, 1958.
- 143. MUHLBOCK, O. and BOOT, L. M. The mechanism of hormonal carcinogenesis. In: Ciba Foundation Symposium. Carcinogenesis. Mechanisms of Action, p. 83. Boston, 1959. Little Brown & Co.
- 144. OBERLING, C. and GUERIN, M. The role of viruses in the production of cancer. Advances Cancer Res., 2: 353, 1954.
- 145. Viruses as causative agents in cancer. Ann. New York Acad. Sc., 54: 869, 1952.
- 146. Subcellular particles in the neoplastic process. Ann. New York Acad. Sc., 68: 245, 1957.
- BEARD, J. W., SHARP, D. G. and ECKERT, E. A. Tumor viruses. Advances Virus Res., 3: 149, 1955.
- 148. BEARD, J. W. Viruses as a cause of cancer. Am. Scientist, 46: 226, 1958.
- FURTH, J. and METCALF, D. An appraisal of tumor virus problems. J. Chronic Diseases, 8: 88, 1958.
- DiMayorca, G., Eddy, B. E., Stewart, S. E., Hunter, S. W., Friend, C. and Bendich, A.

- Isolation of infectious deoxyribonucleic acid from SE polyoma-infected tissue culture. *Proc. Nat. Acad. Sc.*, 45: 1805, 1959.
- STEWART, S. E., EDDY, B. E., GOCHENOUR, A. M., BORGESS, N. G. and GRUBBS, G. E. The induction of neoplasms with a substance released from mouse tumors by tissue culture. *Virology*, 3: 380, 1957.
- 152. TAYLOR, A. R., BEARD, D., SHARP, D. G. and BEARD, J. W. Nucleic acid of the rabbit papilloma virus protein. J. Infect. Dis., 71: 110, 1942
- 153. Beard, J. W. The fallacy of the concept of virus "masking": a review. *Cancer Res.*, 16: 279, 1956.
- 154. Lederberg, J. Viruses, genes, and cells. *Bact. Rev.*, 21: 133, 1957.
- 155. JACOB, F. and WOLLMAN, E. L. Genetic aspects of lysogeny. In: McElroy, W. D. and Glass, B. The Chemical Basis of Heredity, p. 468. Baltimore, 1957. Johns Hopkins Press.
- 156. Kornberg, A., Zimmerman, S. B., Kornberg, S. R. and Josse, J. Enzymatic synthesis of DNA. vi. Influence of bacteriophage T₂ on the synthetic pathways in host cells. *Proc. Nat. Acad. Sc.*, 45: 772, 1959.
- SOMERVILLE, R., EBISUZAKI, K. and GREENBERG,
 G. R. Hydroxymethyldeoxycytidylate kinase formation after bacteriophage infection of E. coli. Proc. Nat. Acad. Sc., 45: 1240, 1959.
- 158. Epstein, M. A. and Holt, S. J. Observations on the Rous virus: integrated electron microscopical and cytochemical studies of fluorocarbon purified preparations. *Brit. J. Cancer*, 12: 363, 1958.
- RUBIN, H. and TEMIN, H. M. Infection with the Rous sarcoma virus in vitro. Fed. Proc., 17: 994, 1958
- 160. Bernhard, W., Bonar, R. A., Beard, D. and Beard, J. W. Ultrastructure of viruses of myeloblastosis and erythroblastosis isolated from plasma of leukemic chickens. *Proc. Soc. Exper. Biol. & Med.*, 97: 48, 1958.
- 161. Levine, M. The cytology of the tumor cell in the Rous chicken sarcoma. Am. J. Cancer, 36: 276, 386, 581 and 37: 69, 1939.
- 162. Dмосноwsкi, L. The milk agent in the origin of mammary tumors in mice. Advances Cancer Res., 1: 103, 1953.
- 163. Andervont, H. B. Genetic, hormonal and age factors in susceptibility and resistance to tumor inducing viruses. Texas Rep. Biol. & Med., 15: 462, 1957.
- 164. Burmester, B. R. Routes of natural infection in avian lymphomatosis. Ann. New York Acad. Sc., 68: 487, 1957.
- 165. Gross, L. Viral etiology of "spontaneous" mouse leukemia: a review. Cancer Res., 18: 371, 1958.
- 166. GROSS, L. Studies on the nature and biological properties of a transmissible agent causing leukemia following innoculation into newborn mice. Ann. New York Acad. Sc., 68: 501, 1957.
- CLAUDE, A. and MURPHY, J. B. Transmissible tumors of the fowl. *Physiol. Rev.*, 13: 246, 1933.
- 168. BEARD, J. W. Etiology of avian leukosis. Ann. New York Acad. Sc., 68: 473, 1957.
- 169. GIERER, A. and SCHRAMM, G. Infectivity of ribo-

- nucleic acid from tobacco mosaic virus. Nature, London, 177: 702, 1956.
- 170. COLTER, J. S. Nucleic acid as the carrier of viral activity. *Progr. M. Virol.*, 1: 1, 1958.
- 171. LATARJET, R. Carcinogenesis by leukemic cell free extracts in mice. In: Ciba Foundation Symposium Carcinogenesis. Mechanism of Action, p. 274. Boston, 1959. Little, Brown & Co.
- 172. Harel, J., Huppert, J., LaCour, F. and LaCour, J. Tumeurs malignes transmissible de la souris, provoquées par injection de preparations contenant de l'acide ribonucleique. *Bull. Assoc. franç. étude cancer*, 1: 74, 1959.
- 173. BIELKA, H., GRAFFI, A. and KRISCHKE, W. Zur Frage der Beteiligung von Nucleinsauren an der chemischen Zusammensetzung des leukamogenen Agens aus filtrierbaren Mausetumoren. Naturwissenschaften, 44: 381, 1957.
- 174. PASCHKIS, K. E., CANTAROW, A. and STASNEY, J. Induction of neoplasms by injection of tumor chromatin. J. Nat. Cancer Inst., 15: 1525, 1955.
- 175. HAYES, E. F., SIMMONS, N. S. and ВЕСК, W. S. Induction of mouse leukemia with purified nucleic acid preparations. *Nature*, *London*, 180: 1419, 1957.
- 176. MEEK, E. S. and HEWER, F. F., An intestinal carcinoma in mice following injection of herring sperm deoxyribonucleic acid. *Brit. J. Cancer*, 13: 121, 1959.
- 177. SHIVE, W. and SKINNER, C. G. Metabolic antagonists. Ann. Rev. Biochem., 27: 643, 1958.
- 178. SKIPPER, H. E. and BENNETT, L. L., JR. Biochemistry of cancer. Ann. Rev. Biochem., 27: 137, 1958.
- 179. Krakoff, I. H. Mechanisms of drug action in leukemia. Am. J. Med., 28: 735, 1960.
- 180. Woods, D. D. The relation of p-amino benzoic acid to mechanism of action of sulfanilamide. *Brit. J. Exper. Path.*, 21: 74, 1940.
- 181. Park, J. T. and Strominger, J. L. Mode of action of penicillin. *Science*, 125: 99, 1957.
- 182. DIXON, M. and WEBB, E. C. Enzymes. New York, 1958. Academic Press.
- 183. COHEN, G. N. and MONOD, J. Bacterial permeases. Bact. Rev., 21: 169, 1957.
- 184. JACQUEZ, J. A. Active transport of β-diazoacetyl-Lserine and 6-diazo-5-oxo-L-norleucine in Ehrlich ascites carcinoma. Cancer Res., 17: 890, 1957.
- 185. SARTORELLI, A. C., LEPAGE, G. A. and MOORE, E. C. Metabolic effects of 6-thioguanine. I. Studies on thioguanine-resistant and sensitive Ehrlich ascites cells. Cancer Res., 18: 1232, 1958.
- 186. Law, L. W. Differences between cancers in terms of evolution of drug resistance. Cancer Res., 16: 698, 1956.
- 187. Nichol, C. A. Studies on resistance to folic acid antagonists. In: Rebuck, J. W., Bethell, F. H. and Monto, R. W. The Leukemias: Etiology, Pathophysiology and Treatment, p. 583. New York, 1957. Academic Press.
- Ciba Foundation Symposium on Drug Resistance in Micro-Organisms. Boston, 1957. Little, Brown & Co.
- 189. Huennekens, F. M. and Osborn, M. J. Folic acid coenzymes and one-carbon metabolism. *Advances Enzymol.*, 21: 369, 1959.
- 190. FRIEDKIN, M. and ROBERTS, D. Conversion of uracil

- deoxyriboside to thymidine of deoxyribonucleic acid. J. Biol. Chem., 220: 653, 1956.
- 191. Сонь, S. S. Unbalanced growth and death: a study in thymine metabolism. In: GRAFF, S. Essays on Biochemistry, p. 77. New York, 1956. John Wiley & Sons, Inc.
- 192. DEMARS, R. and HOOPER, J. L. A method of selecting for auxotrophic mutants of hela cells. J. Exper. Med., 111: 559, 1960.
- 193. FARBER, J., TOCH, R., SEARS, E. M. and PINKEL, D. Advances in chemotherapy of cancer in man. Advances Cancer Res., 4: 1, 1956.
- 194. HERTZ, R., BERGENSTAL, D. M., LIPSETT, M. B., PRICE, E. B. and HILBISH, T.F. Chemotherapy of choriocarcinoma and related trophoblastic tumors in women. Ann. New York Acad. Sc., 80: 262, 1959.
- 195. 6-Mercaptopurine. Ann. New York Acad. Sc., 60: 183, 1954.
- 196. LUKENS, L. N. and HERRINGTON, K. A. Enzymatic formation of 6-mercapto-purine ribotide. Biochem. et biophys. acta, 24: 432, 1957.
- 197. LIEBERMAN, I. and OVE, P. Enzyme studies with mutant mammalian cells. J. Biol. Chem., 235: 1765, 1960.
- 198. Buchanan, J. M., Levenberg, B., Melnick, I. and HARTMAN, S. C. The specific action of azaserine on enzymes concerned with purine biosynthesis. In: REBUCK, J. W., BETHELL, F. H. and MONTO, R. W. The Leukemias: Etiology, Pathophysiol-

- ogy and Treatment, p. 523, New York, 1957. Academic Press.
- 199. KAMMEN, H. O. and HURLBERT, R. B. Amination of uridine nucleotides to cytidine nucleotides by soluble mammalian enzymes: role of glutamine and guanosine nucleotides. Biochim. et biophys. acta, 30: 195, 1958.
- 200. MATTHEWS, R. E. F. Biosynthetic incorporation of metabolite analogues. Pharmacol. Rev., 10: 359,
- 201. Brockman, R. W., Bennett, L. L., Simpson, M. S., WILSON, A. R., THOMSON, J. R. and SKIPPER, H. E. A mechanism of resistance to 8-azaguanine. II. Studies with experimental neoplasms. Cancer Res., 19: 856, 1959.
- 202. MAZIA, D. The life history of the cell. Am. Scientist, 44: 1, 1956.
- 203. Moeschlin, S. Round table discussion on therapy. In: REBUCK, J. W., BETHELL, F. H. and MONTO, R. W. The Leukemias: Etiology, Pathophysiology and Treatment, p. 645. New York, 1957 Academic Press.
- 204. Goldthwait, D. A. In: Rebuck, J. W., Bethell, F. H. and Monto, R. W. The Leukemias: Etiology, Pathophysiology and Treatment, p. 555. New York, 1957. Academic Press. 205. Endicott, K. M. The national cancer chemother-
- apy program. J. Chronic Dis., 8: 171, 1958.
- 206. Cohen, G. N. and Gros, F. Protein biosynthesis. Ann. Rev. Biochem., 29: 525, 1960.

Arthralgias, Thrombophlebitis, Weight Loss, Pleural Effusion and Elevated Alkaline Phosphatase

S TENOGRAPHIC reports, edited by Lillian Recant, M.D. and W. Stanley Hartroft, M.D., of weekly clinicopathologic conferences held in the Barnes and Wohl Hospitals are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

A THIRTY year old white optometrist was admitted to the Barnes Hospital for the first time on January 29, 1960, with the complaint of pain in the hips of seven months' duration. He died on March 13, 1960, forty-five days later.

On two separate occasions during his child-hood diagnoses of "acute rheumatic fever" and "ulcerative colitis" were made. The former was characterized by severe migratory polyarthritis with no known cardiac murmur, manifestations or residua; the latter condition began around the age of nine years, consisted of approximately 8 stools daily, and persisted for about three years. The consistency of the stools at the time was unknown. The patient had enjoyed excellent health subsequently except for an uneventful thyroidectomy which was performed in 1951 for the removal of an apparently asymptomatic "thyroid adenoma."

The present illness began in March 1959, ten months prior to admission, with the onset of a flu-like syndrome (myalgia, headache, earache, mild cough, sore throat and fever). Symptoms subsided over the next two weeks following the administration of Chloromycetin. Several weeks later, a well localized area of tenderness developed on the anterior aspect of the right side of the chest, which rapidly progressed to involve the entire wall on the right side of the chest. It bore no relationship to either respiration or body motion, and subsided spontaneously after several days.

In June 1959, the patient was awakened by the acute onset of pain in the left hip and right shoulder. There was no overt evidence of arthritis; the pain in the shoulder tended to be aggravated by any motion of the right arm and was present variably for only the next month. The pain in the left hip although, relieved slightly by movement of the leg, was followed in several weeks by the onset of pain in the right hip, with both hips being similarly and variably painful up to the time of admission to the Barnes Hospital, seven months later. Likewise, during this month the patient complained of transient episodes of "tenderness" in the right upper quadrant of the abdomen which were unrelated to meals, position, or exertion.

In September 1959, following an interval of general fatigue and somnolence, the patient had the acute onset of fever (temperature of 105°F.), chills, myalgia, abdominal cramping, and nausea. His symptoms had appeared shortly after eating, and his nausea lasted only several hours. His wife, who ate the same food, remained asymptomatic. Subsequently there were nightly febrile episodes together with a marked increase in pain in both hips and an aggravation of chronic purulent postnasal drip. The patient did not respond to therapy with Chloromycetin and was therefore hospitalized. His studies were said to show "pleural thickening," a sedimentation rate of 30 mm. per hour, and "normal" findings in sinus films, hepatic function tests, and heterophil agglutination. Fever disappeared spontaneously during his hospitalization but the patient subsequently noted a loss in weight (approximately 35 pounds during the following three months) as well as severe weakness and atrophy of his left leg. He was hospitalized again in November 1959, two months prior to his admission to the Barnes Hospital, and

shortly thereafter "phlebitis" of his right arm developed, which was characterized by redness, tenderness, heat and palpable superficial "cords." The latex agglutination test was reported as being reactive, with serum electrophoresis demonstrating an increase in the gamma globulin fraction. The sedimentation rate was 60 mm. per hour and tests for the demonstration of L.E. cells were said to have had negative results. Review at Barnes Hospital of the roentgenologic examinations taken at that time were said to show osteoporosis of the left femur, right pleural effusion, hepatomegaly, abnormal neuromotor activity of the small bowel, loss of haustral markings with questionable areas of ulceration in the ascending and transverse colon with otherwise normal evaluations of the spine, pelvis, hips, femur, upper gastrointestinal tract, and intravenous pyelograms. Lumbar puncture was said to have been negative. The patient was treated with Butazolidin,® ACTH, and Medrol® without any apparent improvement in his condition either at the time of discharge or subsequently.

In December, "pain in the entire left leg" developed, which was followed by severe edema of the entire leg with redness and heat. The patient was hospitalized, treated with bed rest, antibiotics, and Dicumarol, and his condition improved. Except for a painless episode of mild shortness of breath his hospitalization was uneventful; he was discharged on December 31.

Two weeks prior to his admission to the Barnes Hospital, the patient was awakened by the sudden onset of severe shortness of breath, which was relieved by sitting up. He was hospitalized, and the administration of Dicumarol was discontinued. His right foot was found to be swollen with the veins on his right thigh and abdomen markedly dilated. A large amount of amber fluid was removed from the right side of his chest and studies of this fluid were said to be negative, "showing mesothelial cells." Bone marrow obtained by aspiration was interpreted to be normal. At this time there was the insidious, painless onset of swelling of the entire right leg without any other detectable signs, necessitating the use of ace bandages up to the time of his admission to the Barnes Hospital. Dyspnea, swelling of his right leg, and multiple joint pains, particularly of his left hip, persisted.

The patient's past history was non-contributory. His father has hypertension and, except for a relative who had tuberculosis declared arrested in 1951, the remainder of the family history was also non-contributory.

On physical examination the blood pressure was 150/100 mm. Hg, pulse 96, respirations 18, and temperature 37.4°c. The patient was a well developed, well nourished white man, very observant, cooperative, sitting up in bed, slightly short of breath, with evidence of recent weight loss. The left lobe of the thyroid was palpable; the neck veins were not distended. There was limited expansion of the entire right thorax together with absent breath sounds. dullness and decreased tactile fremitus to the level of the second rib anteriorly. Decreased breath sounds, dullness, and decreased fremitus were detected at the base of the left lung. No mediastinal shift was demonstrated. The left border of cardiac dullness was 1 cm. to the left of the mid-clavicular line in the fifth intercostal space. The pulmonic second heart sound was louder than the aortic and was described as being split, as was the mitral first heart sound, which was also accentuated. There was an apical grade 2/6 systolic murmur heard also along the left sternal border. The liver edge was firm, non-tender, and palpable 2 to 3 cm. below the right costal margin. Two thrombosed external hemorrhoids were found. The right leg was swollen distal to the inguinal ligament; all the muscle groups of the left leg were atrophic. Measurements of the legs revealed the right to be larger by 7.5 cm. at the mid-calf region and 10.5 cm. at the mid-thigh. The right leg also demonstrated 2 plus pitting edema, decreased temperature, tenderness of the calf and a mild ruddy rubor of the entire leg, which was aggravated by hanging the leg downward. There was moderate pain on external rotation of both hips, greater on left, as well as definite left quadriceps and gastrocnemious muscle weakness. All peripheral pulses were strong and equal.

The laboratory data were as follows: The red blood cell count was 5,050,000 per cu. mm., hemolgobin 13.5 gm. per cent, packed cell volume 38 per cent, white blood cell count 15,400 per cu. mm., platelet count 250,000 per cu. mm., corrected sedimentation rate 18 mm. per hour, with a differential of 68 segmented forms, 7 per cent bands forms, 22 per cent lymphocytes and 3 per cent monocytes. The reticulocyte count was 5 per cent (repeat value was 0.9 per cent on February 1). The urine specific gravity was 1.018, pH 4.5, the albumin and sugar reaction was negative, with 15 to 20

white blood cells per high power field in the centrifuged specimen. The results of three subsequent urinalyses were within normal limits. The blood cardiolipin reaction was negative. The stool examination on admission was negative for occult blood and neutral fat, as were three subsequent examinations. The C-reactive protein was 2 plus on two determinations, while the antistreptolysin O titer was greater than 500 units initially, and 316 units five days later. Negative agglutination results were reported for the Coombs' tests, heterophil, brucella, salmonella groups, and the latex test. Three examinations of the blood for the detection of L.E. cells were negative. A non-diagnostic serum electrophoretic pattern was obtained which demonstrated a slight decrease in the gamma globulin fraction. The blood uric acid was 7.4 mg. per cent (repeat values were 5.3 mg. per cent and 5.6 mg. per cent), amylase of less than 50 units on two occasions (final value was 76 units on March 1), alkaline phosphatase 28.4 Bodansky units, calcium 10.9 mg. per cent, and phosphorus 5 mg. per cent. Normal values were reported for the fasting blood sugar, blood urea nitrogen, serum proteins, bilirubin, cephalin-cholesterol flocculation, prothrombin time, cholesterol, serum glutamic oxaloacetic and pyruvic transaminases, cholesterol and creatinine.

The electrocardiogram was interpreted as showing an abnormal form of ventricular complex on the basis of changes in the S-T segment and T wave. Roentgenograms of the chest were interpreted as showing a massive right pleural effusion, with interval increase since December, and questionable left ventricular enlargement. Barium enema examination and small bowel series revealed hepatosplenomegaly, a relatively ahaustral colon with changes compatible with chronic inflammatory disease, and findings compatible with a neuromotor disturbance of the small intestine. The sigmoidoscopic examination was described as being within normal limits.

The administration of analgesics successfully controlled the patient's pain and a thoracentesis on the right side yielded 500 cc. of serosanguinous fluid, which did not clot and which had a specific gravity of 1.013. There was no growth reported on routine, acid-fast bacilli and fungal cultures of the fluid, and no diagnosis was made on examination of the cell block. The patient's symptoms remained unchanged and there were

late afternoon spikes in temperature, ranging around 38.5° to 38°c. on January 31. The throat was cultured on two occasions, revealing each time a moderate growth of hemolytic, coagulase positive, yellow staphylococcus, whereas the initial culture showed only a few alpha hemolytic streptococcus in contrast to the heavy growth of both alpha hemolytic streptococcus and neisseria reported on the culture obtained five days later. A repeat thoracentesis on February 3 vielded 1,100 cc. of fluid, negative cytological and cultural studies. There were positive skin reactions to histoplasmin and intermediate strength P.P.D. (negative to coccidiodin and blastomycin), as well as non-diagnostic biopsies of the gastrocnemius muscle and of a thrombosed vein on the dorsum of the foot, except for slight sclerosis in the latter.

The arthralgias and swelling of the right leg increased in severity; the fever persisted. After demonstration of a very rapid thromboplastin generation test and a low plasminogen level, together with normal values for prothrombin time, euglobulin-lysis, fibrinogen, and factor v, the administration of prednisone (80 mg. per day) was begun on February 8. Two days later the patient was afebrile and remained so during the remainder of his hospitalization. There was also marked improvement in the arthralgias and pain, although prominent blanching of the left fingers had concomitantly developed. During the next two weeks, the serum alkaline phosphatase values were in the range of about 31 Bodansky units; the calcium was 10.2 mg. per cent, and phosphorus 3.3 mg. per cent. There was 11.2 per cent retention of the bromsulphalein dye after forty-five minutes although results of repeat liver profile tests remained within normal limits. On February 10 films of the chest demonstrated an interval increase in the massive right pleural effusion and in the size of the left intrapulmonary vessels without significant change in the appearance of the cardiac silhouette; dullness and decreased breath sounds were detected over this area as well as tubular breathing above the area of dullness. Despite repeated failures at thoracentesis, the chest findings remained subsequently unchanged although there was apparently some lessening in the degree of dyspnea. On February 12 physical therapy was initiated with progressive improvement subsequently. On February 22 it was noted that a smooth, slightly tender liver edge was felt 7 cm. below the right costal

margin; the size of the patient's leg and the dilated abdominal veins had decreased. At this time the cephalin-cholesterol flocculation was 4 plus (repeat value on March 1 was normal), thymol turbidity 1.4 units, albumin 4 gm. per cent, globulin 2.3 gm. per cent.

On February 26 the dose of prednisone was reduced; two days later the patient noted the onset of recurrent pain in the back and in the lower right quadrant of his abdomen as well as generalized weakness and subsequently the appearance of severe pain in both hips. At this time, his weight loss since admission had reached 16 pounds, his alkaline phosphatase had reached a peak of 44.5 Bodansky units, calcium 9.8 mg. per cent, phosphorus 3.7 mg. per cent. There were five Sulkowitch tests performed on the urine, trace reactions being reported three times, 1 plus on one occasion and 4 plus on the final determination. On March 3 the prothrombin time was 48 per cent of normal, the hematocrit was 43 per cent, and two positive stool guaiac reactions were reported without any other evidence of bleeding. The patient was given Mephyton® and on March 5 he underwent an uneventful exploratory laparotomy. He died on March 13.

CLINICAL DISCUSSION

Dr. Carl V. Moore: The patient, an optometrist thirty years of age, had two serious illnesses during childhood, one thought to be acute rheumatic fever, and the other ulcerative colitis. The latter persisted for about three years before remission occurred. The present illness probably began as an infection of the upper respiratory tract in March 1959. Several weeks later he had tenderness localized to the right anterior part of his chest, not related to respiration. The discomfort spread over the entire right side of his chest but subsided spontaneously within a few days. His clinical course thereafter was characterized by fever, weight loss, weakness, joint pains, recurrent thrombophlebitis, right pleural effusion, abdominal and back pain, an enlarging liver, and unimpressive response to steroid therapy. There was no clear evidence of cardiac or renal involvement. An exploratory laparotomy was performed on March 5, 1960; he died one week later. The most striking physical signs were those of right pleural effusion, hepatomegaly, weakness, and questionable atrophy of the muscles of the left

thigh and leg. There was edema of the right leg, with dilated veins on the right thigh and abdomen, and palpable thrombosed veins. Deep tendon reflexes and the plantar responses were normal. Pleural fluid had the characteristics of a serosanguineous transudate. The uric acid was slightly elevated on several occasions. The bromsulphalein retention was 11 per cent; the alkaline phosphatase was strikingly elevated; results of the cephalin-cholesterol flocculation test were negative several times, positive once. The antistreptolysin titer was elevated; the reaction to skin tests for tuberculosis and histoplasmin, and the latex agglutination was positive. A barium enema examination was interpreted as possible ulcerative colitis. Dr. Brown, will you discuss the roentgenograms?

DR. MARK BROWN: This patient had an extensive roentgenological work up, both at the referring hospital and later at the Barnes Hospital. The first chest film available to us was taken on November 16, 1959. It showed an elevated right leaf of the diaphragm, minimal right pleural effusion, and some slight discoidatelectasis on the right. The pulmonary vessels were full but not necessarily abnormal. The next film was taken shortly before the patient's discharge from the other hospital (December 26, 1959). It showed the increase in the right pleural effusion without much other change. On the next film, which was taken shortly after the patient's admission here, the right pleural effusion was further increased. There was no contralateral shift of the midline structures, such as one would ordinarily expect with an effusion as extensive as this, and one suspects an underlying atelectasis hidden by the effusion. The next film, which was taken approximately eleven days later, showed an increase in the right pleural effusion, with increased vascularity on the contralateral side. The last film was taken approximately three weeks before the patient's death and showed no additional change.

Review of the upper gastrointestinal series and films of the spine, leg, and knee made at the other hospital were not significantly abnormal. The pyelograms performed on November 18, 1959, showed normal renal contours and a symmetrical collecting system. We had an unusually good look at the ureters without any evidence of displacement. The barium enema examination carried out at the other hospital was very interesting. The most significant

changes were seen in the evacuation film which was characteristic of ulcerative colitis. There was, in addition, a fusiform area of constrictual narrowing in the ascending colon demonstrated on all the films of two different examinations.

DR. MOORE: Dr. Smith, the right anterior chest pain was one of the first symptoms. Later pleural effusion, possible pulmonary infarcts associated with episodes of dyspnea and orthopnea, and a dilated left pulmonary artery developed. Would you tell us how you explain these manifestations and put them together?

Dr. John R. Smith: The chest pain was initially localized to one area of the right side of the chest, but gradually involved the entire right side of the chest. It bore no relationship to respirations, or to body motion, and subsided spontaneously after a few days. Visceral pain arising in the chest is usually recognizable; it is generally sufficiently characteristic so that one can tell that it is visceral pain. I speak now particularly of cardiac pain, of the pain of pleuritis, of esophageal pain, or the pain of mediastinitis. Chest wall pain from inflammatory disease or mechanical disease, or from trauma is usually accompanied by localized areas of tenderness, since the structures of the chest wall which are subject to a good deal of motion on ordinary movement of the body, and particularly upon respiration, are involved. This pain was not chest wall pain. The description is a little sketchy. The origin of the pain may have been in the spine, and this patient may have had a form of referred pain which can manifest itself by remaining localized or involving large areas, not necessarily responding to motion of the chest or to respiration. One wonders whether there may have been some irritative factor in or about the spinal nerves as they emerge from the spine—a mechanical irritation of some sort, perhaps from splinting of the spine; from general discomfort. There also may have been localized infection of some type, or interference with the blood supply of the nerves. I suppose one should include possible invasion of the spinal nerves by viruses, although the chicken pox-like virus invasion of spinal nerves and peripheral nerves (herpes zoster) is the best known and by far the most severe.

There was pleural effusion, which certainly was striking. Of the causes of pleural effusion that come to mind the two most common are (1) interference with blood flow through the lungs, as in congestive heart failure, and

infarction of the lungs and (2) infection of the lung. There are many less common causes.

The patient became debilitated over a period of time, which suggests that pulmonary emboli and infarction of the lung, with or without infection may have been present.

DR. MOORE: Extensive involvement of the pulmonary vessels on the right could account for the terminal increase in the size of the left pulmonary vessels, could it not?

Dr. Smith: Yes, because that would mean the entire blood volume as moved by the heart would be shunted to one lung.

Dr. Moore: Dr. Karl, how would you interpret the high alkaline phosphatase values, and the moderate retention of bromsulphalein in the face of other relatively normal hepatic function tests, like the cephalin-cholesteral flocculation?

Dr. Michael M. Karl: One single positive reaction to the cephalin-cholesteral flocculation test with a number of others negative, and bromsulphalein retention of 11 per cent in a patient who is running fever at the time certainly is not impressive evidence of diffuse hepatocellular disease. The elevated alkaline phosphatase on the other hand is certainly very significant. Elevations of this degree are uncommon in diffuse hepatocellular disease, but do occur in extrahepatic obstruction of the biliary tree, in intrahepatic cholestasis such as is seen in primary biliary cirrhosis, in cholangiolitic hepatitis, and in association with certain drug hypersensitivities such as with Thorazine.® Elevations of this degree also occur in focal granulomatous lesions involving the liver, such as tuberculosis, in sarcoid, amyloidosis, Hodgkin's disease, and occasionally disseminated lupus. Lastly, such elevated alkaline phosphatase values occur in space-occupying lesions of the liver such as carcinoma of the liver, primary or metastatic, and in liver abscess. These are the more common conditions associated with elevations of the alkaline phosphatase.

DR. MOORE: In this instance the first three can pretty well be eliminated on the basis of the history and the absence of jaundice; the last two, the granulomatous and the space-occupying lesions, such as carcinoma, would seem to be the two that cannot be ruled out. Would you agree?

DR. KARL: I would certainly agree that these are the only two that merit consideration.

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DR. MOORE: Four diagnoses seemed most likely to the people who cared for this man in the hospital: (1) a "collagen disease," either lupus or polyarteritis, or a hypersensitivity vasculitis, (2) ulcerative colitis with carcinoma of the colon, and possible metastasis to the liver, (3) visceral thrombophlebitis, (4) some kind of infectious granuloma. Dr. Harrington, would you discuss the first of these?

DR. WILLIAM J. HARRINGTON: This man had, in addition to the arthritis and arthralgia mentioned, fever, pleuritis, and possible myocardial and endocardial disease. It might also be mentioned that ulcerative colitis is a rare but not unheard of event in classic lupus erythematosus. However, only one of 118 patients with systemic lupus erythematosus whose records were recently reviewed at Presbyterian Hospital,* and only four of 138 patients at Johns Hopkins† had ulcerative colitis. So the history of ulcerative colitis would not strongly support the diagnosis of systemic lupus erythematosus. In addition, the patient's sex is wrong; better than 75 per cent of the patients with lupus are women. The syndrome started at a somewhat younger age in this patient than would be usual in systemic lupus erythematosus. One might also point out there were no skin lesions, no renal involvement, no hematologic derangements, no eye ground changes, no psychotic or neurologic manifestations or other findings common in systemic lupus. There were no L.E. cells found. And finally, if one selects a symptom the man did have, the pulmonary involvement was unilateral which would be unusual in this disease. The evidence for lupus erythematosus is too flimsy to make this diagnosis tenable.

As far as other collagen diseases are concerned, polyarteritis is a possibility, but there is no substantial supporting evidence. The type of neurologic disease he may have had would be atypical. Nor is there good evidence for angiitis of any type. Particularly notable is the absence of evidence of renal disease.

I believe quite strongly that the evidence for autoimmune disease of any kind is minimal, unless one wishes to accept the present evidence that ulcerative colitis itself is a form of autoimmune disorder.

DR. MOORE: Dr. Vavra, about two months ago you impressed me at one of these conferences by making a correct diagnosis of visceral migrating thrombophlebitis. Is that a likely possibility here?

Dr. John Vavra: There is no doubt that this patient had a migratory thrombophlebitis. The important question is whether this was the "visceral type" in which no underlying disease will be found. Visceral migratory thrombophlebitis is a disease involving veins, and in very few instances arteries are involved. Patients with this disease complain of symptoms, which differ widely depending on the location of the involved veins and the rapidity of development of the thrombotic episode. In about half of the patients, the chief manifestation is recurrent peripheral thrombophlebitis, complicated by repeated large and small pulmonary infarcts. Eventually when thrombophlebitis or thromboses extend to deeper veins, particularly in the abdomen, the illness becomes more serious and the course is accelerated. Our patient certainly showed evidence of a peripheral thrombophlebitis. In November it was definite in the right arm; December in the left leg; and in January in the right leg. In addition, our patient also probably had episodes of pulmonary infarction. What is the evidence that our patient had a visceral or deep vein thrombophlebitis? The evidence for deep vein thrombophlebitis is much less convincing. He had episodes of abdominal pain, an enlarged liver, and abnormal hepatic function studies, which might be suggestive of thromboses either occurring in small radicals of the hepatic vein, or in other veins in the abdomen. He had dilated veins over the abdominal wall, suggestive of deep vein thromboses. If the inferior vena cava were thrombosed, the thrombosis must have been below the renal veins, since the kidneys apparently were not involved. If the thromboses were in the abdomen, they probably did not involve the mesenteric vessels very extensively since the patient did not have the severe symptoms and gastrointestinal bleeding one often sees under those circumstances. He might have had small thromboses in the hepatic vessels; the liver function studies are compatable except for the markedly elevated alkaline phosphatase. Two of the primary symptoms the patient complained

^{*} Combined Staff Clinic (Columbia University College of Physicians and Surgeons). Systemic lupus erythematosis. Am. J. of Med., 28: 416, 1960.

[†] Harvey, A. M., Stulman, L. E., Tumulty, P. A., Carley, C. L. and Schowich, R. H. Systemic lupus erythematosis. Review of the literature and critical analysis of 138 cases. *Medicine*, 33: 291, 1959.

of were arthralgia and myalgia. These are uncommon symptoms in visceral migrating thrombophlebitis, however a few patients have complained of severe hip pain, and on roent-genographic examination have shown destructive lesions and osteoporosis, suggesting vascular occulsion in bones. I have no information as to whether the alkaline phosphatase was elevated or what the actual bony changes were in these patients.

In summary, I think our patient showed definite peripheral migratory thrombophlebitis, but questionable deep vein thrombophlebitis. The other features difficult to explain by this diagnosis are ulceration of the colon, muscular atrophy and myalgia, the prominent joint symptoms, and the elevated alkaline phosphatase.

DR. MOORE: Dr. Fletcher, would you interpret the coagulation studies? Specifically, is there any evidence of a hypercoagulable state? If your answer is "yes," does that help us with

the differential diagnosis?

Dr. Anthony Fletcher: One of the most disappointing features of research in blood coagulation, as related to the clinical field, has been the absence of any definite correlation between increase of any specific blood coagulation factors and the incidence of clinical thrombosis. When the thromboplastin generation test came out, it was thought that this might be a good test for the detection of thrombosing tendencies or more specifically accelerated clot formations. In actual fact, the thromboplastin generation test did not prove very valuable in this area. However, recently at the Mayo Clinic* it has been suggested that if the plasma that is used in this test is diluted out then the test might be used to detect clot accelerating factors. Such a test was performed on this patient. Very simply, what we found was this: If the test conditions were set up so that normal plasma produced sufficient thromboplastin to cause a clotting time in fourteen minutes, this patient's plasma produced sufficient thromboplastin in four minutes. This is a grossly accelerated figure and quite outside what we would believe would be the normal type of response. The only evidence tying this test in with an increased

thrombosing tendency also comes from the Mayo Clinic. Some seventy patients with various types of arterial and venous occulsive disease were studied as early in the course of their disease as possible. In one-third of these patients the result of this test was found to be positive. That is, in one-third of these patients with clinical evidence of a thrombotic tendency the test result was abnormal and there was evidence of a clot accelerating tendency in the patient's plasma. Interpretation is obviously difficult. One of the difficulties has been that in patients in whom abnormalities of the coagulation system have been found, there has been a very poor correlation with the clinical state. An attempt to evaluate this type of laboratory test has recently been made in animals using the well known serum thrombosis technique. This technique involves the production of a thrombosis in a stagnant area of blood when serum is injected. Now this type of correlation with the in vitro and in vivo test has not yet been attempted with a modified thromboplastin generation test, and until it is I think we should use much caution in the interpretation of these tests.

DR. Moore: The weakness and possible atrophy of the muscles of the left leg are difficult to explain on a neurologic lesion in the presence of normal deep tendon reflexes, and in the absence of any sensory changes. Unilateral peripheral neuritis seems quite unlikely. The changes are more likely due to disuse atrophy and weakness in the left side, the disuse because

of pain.

This man as a child was thought to have ulcerative colitis, with symptoms for approximately three years. When symptoms during an acute attack last for more than six months, prolonged remissions are rare. In this instance, however, there was a sustained remission. We know that remissions can last for many years, and that during the remissions the colon may demonstrate the abnormal changes of ulcerative colitis, even while the patient is asymptomatic. Here, however, we have to reckon with the fact that the remission was eighteen years in duration; and that even during the terminal illness the patient had no diarrhea, although a few stools contained blood. If we also postulate that a carcinoma of the colon developed which metastasized to the liver, and postulate that the carcinoma represented a complication of ulcerative colitis, then we must assume that the lesion was active during the period of remission, even though there were

^{*} Spittel, J. A., Pascuzzi, C. A., Thompson, J. H. and Owen, C. A. Acceleration of early stages of coagulation in certain patients with occlusive arterial and venus diseases; use of a modified thromboplastin generation test to evaluate clot acceleration. *Proc. Soc. Mayo Clin.*, 35: 37, 1960.

no symptoms of any kind. Lastly, we have to reckon with the fact that a sigmoidoscopic examination revealed no abnormalities.

Dr. Gieselman, do you think this patient had ulcerative colitis? If so, would you discuss the various problems I have raised?

DR. RALPH GIESELMAN: From the roentgenographic evidence, it is reasonable to say that this man had idiopathic ulcerative colitis, involving at least the proximal half of his colon and probably also the last few inches of his ileum. The natural history as exhibited in this case is a little unusual. Ulcerative colitis, beginning presumably at age ten, usually involves in the course of the disease the entire colon and tends to run a more hectic course than in the adult patient, but clinical remissions do, of course, occur. The fact that this patient had very few symptoms after the early years would be unusual, especially in this group. Now I am sure that even without symptoms of active ulcerative colitis, as manifested by bloody diarrhea, the disease can be quite active with x-ray evidence of involvement quite out of keeping to what is presented clinically. The patient could have had the disease then for twenty years with varying degrees of activity. We know that carcinoma of the colon is most commonly seen in just this type of person with early onset and long duration of the disease. Often of course, the patients have not run a hectic course or else they would have come to surgery long before. It is usually very difficult to make a diagnosis of carcinoma of the colon in these people because the roentgenographic changes are atypical, and may easily be confused with stricture or diffuse narrowing, as in this case. Two of the cases diagnosed at this hospital were recognized at surgery. Certainly, therefore, this man could have such a lesion; many of his clinical manifestations were those seen in ulcerative colitis, and/or carcinoma with metastasis.

DR. MOORE: One certainly can have migrating thrombophlebitis in as many as 10 per cent of patients with ulcerative colitis. Arthralgia and arthritis are common. Pulmonary infarcts may occur, and with the pulmonary infarcts, pleural effusion. Hepatic lesions may be either cirrhosis or metastasic.

Dr. Davidson, many people are coming to regard ulcerative colitis as a disease caused by or associated with immune mechanisms. Perhaps the relationship between ulcerative colitis and diseases like systemic lupus erythematosus is not as remote as we formerly thought. Would you discuss these possible relationships?

Dr. John Davidson: People originally investigated this aspect of ulcerative colitis largely because many extra colonic manifestations of the disease are similar to those found in diseases regarded as having an autoimmune, or an allergic pathogenesis. Kirsner and his associates were able to produce experimental colitis in rabbits which had been sensitized to egg albumin by repeatedly challenging these animals after their colons had non-specifically been irritated with dilute formaldehyde enemas.* In this way, these investigators were able to stimulate a significant antigen-antibody reaction, largely confined to the colon; a necrotizing and hemorrhagic colitis was produced which had some of the aspects of ulcerative colitis as we see it in man. More recently, a series of experiments published by Broberger and Perlman from Sweden described an antibody in the serum of twenty-eight of thirty children with ulcerative colitis.† This antibody would react with an extract of human colonic tissue, and the antigen appeared to be a polysaccharide. The authors believed that this was a true antibody, since it migrated with the gamma globulin fraction on electrophoresis. One wonders if this antibody is really responsible for engendering the pathologic process, or is merely an immunologic by-product of the long-standing altered physiology in the colon? Broberger and Perlman were able to demonstrate the adsorption of the antibody on human colon cells growing in tissue culture by the fluorescence technic. No cytotoxic effects on these cells were observed, even when they were well coated with the antibody. In general then, the colon can be sensitized and a colitis can be produced; also, an antibody has been found in the serum of patients with ulcerative colitis which will react with colonic tissue. It should be emphasized however, the experimental colitis can always be readily differentiated from the disease in man, and there is no proof that the antibody which has been found might not merely be an artifact of long-standing disease.

DR. MOORE: Dr. Shatz, do you have any explanation for the ulceration in the colon, other than the ones already mentioned?

^{*} Kirsner, J. B. and Elchlepp, J. The production of an experimental ulcerative "colitis" in rabbits. *Tr. A. Am. Physicians*, 70: 102, 1957.

[†] Broberger, O. and Perlman, P. Autoantibodies in human ulcerative colitis. J. Exper. Med., 110: 657, 1959.

DR. BURTON SHATZ: I do not think that we are justified in making a diagnosis of chronic idiopathic ulcerative colitis in this patient. The absence of a clinical history of relapse characterized by bloody diarrhea since his disease in childhood is certainly unusual. The location of the lesion on the right side of the colon with no rectal envolvement is atypical. The terminal event with no diarrhea makes diagnosis of ulcerative colitis difficult. There is x-ray evidence of ulceration of the cecum, ascending colon, and perhaps the transverse colon, and the terminal ileum. Other causes for this lesion that should be considered are tuberculosis and amebic infection. There are no reports of examination of the stools for parasites; so that we do not know whether amebas were present. However, the roentgenographic findings are compatable with an amebic infection. The first chest film showed an elevated right diaphragm which is suggestive of an enlarged liver. The following chest films showed a right pleural effusion which could have been due to inflammatory disease coming from below the diaphragm. Also, the elevated alkaline phosphatase and the low serum diastase are compatible with an amebic abscess. However, there are other things which we cannot explain. such as arthralgias, thrombophlebitis, and so on, which are against the diagnosis. Tuberculosis could, perhaps, explain everything this patient had. The location of the lesion in the gastrointestinal tract and the migratory arthralgias could go along with tuberculosis. The pleural effusion could be explained. The disturbances in hepatic function, as Dr. Karl has pointed out, are compatible with granulomatous involvement of the liver such as one could see with tuberculosis. Both these diseases should be strongly considered, but I would favor tuberculosis.

DR. CARL H. HARFORD: The general designation of granulomatous diseases should include fungus infections.

Dr. Moore: Sputum, blood, and pleural fluid were all cultured for fungi; no growth was obtained.

My primary diagnosis would be carcinoma of the colon, as a complication of long-standing ulcerative colitis, with metastases to the liver, and with lesions secondary to migrating thrombophlebitis.

Dr. Walsh, will you tell us what was found at laparotomy?

DR. JAMES W. WALSH: The liver was very

grossly enlarged and studded with hard, white nodules. There were also tumor implants in the peritoneum. Postoperatively the patient's course was identical to that preoperatively. The prednisone dosage was decreased and he was given 2.4 gm. of Cytoxan® without any noticeable effect. On March 11 he had moderate shortness of breath without any chest pain, and on the morning of March 13 was found to have died during his sleep.

Dr. Moore: Dr. Saltzstein, would you discuss the slide that was taken at laparotomy?

DR. SIDNEY L. SALTZSTEIN: We actually received, as the protocol states, three different specimens. One was pleural fluid with nothing but normal mesothelial cells in it. The second was a biopsy specimen of muscle and a thrombosed vein. The muscle biopsy specimen was normal. The thrombosed vein was an ordinary organizing thrombus. The wedge of liver removed at laparotomy showed two nodules of carcinoma. Quite a dense fibrous stroma surrounded large tumor cells which tended to form small acini or duct-like spaces. We believe that this is a primary cholangiolitic carcinoma, rather than metastatic carcinoma. There is one thing which already has been mentioned that I would like to emphasize. A carcinoma which arises in ulcerative colitis may not only present no specific roentgenographic findings, but it may also present none of the usual gross findings of carcinoma of the colon. Sometimes the only way we find these cancers in a resected colon, is by examining enough routine sections. Frequently there will be a small area which is slightly firmer than the surrounding tissue. The carcinoma arises between the pseudopolyps, not in or from them.

DR. MOORE: At least we have the carcinoma, but have apparently not diagnosed the primary site correctly.

PATHOLOGIC DISCUSSION

DR. JACQUES CHENARD: Exterior examination of this man showed quite prominent edema of both legs; he also had prominent veins on the lower part of his abdomen and also on his anterior chest wall. On opening the peritoneal cavity about 300 cc. of blood-tinged fluid were found. And in the right pleural cavity about 2,000 cc. of blood-tinged fluid were present. The brain was normal. The right lung showed extensive atelectasis, and the cut surface was grayish, its consistency was very soft and doughy.

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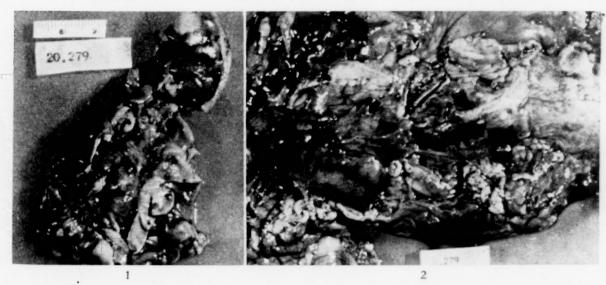


Fig. 1. Gross photograph of the inferior vena cava opened to reveal a gray, friable thrombus-mass completely filling the lumen.

Fig. 2. Portion of the mesentery is shown with a thrombus filling a major mesenteric artery which occupies the upper portion of the center of the field.

It was probably functionless. The pleura over it was very thickened. In the left lung the upper lobe was consolidated and here there was found a recent infarct. Over the pleura of that lung were present small grayish flecks, about 5 mm. in diameter, with small depressed centers which were grossly diagnosed as metastases. A lymph node in the left hilum was calcified and was suspected of containing tumor. There were also some subpleural calcified nodules, but no other gross evidence of tuberculosis.

The liver weighed 4,350 gm. Its right lobe was completely and uniformly involved by tumor. Tumor thrombi were present in many vessels. In the left lobe, the tumor was nodular. The tumor spread from the right lobe of the liver and invaded the right adrenal, so that it was hardly recognizable. Tumor also had extended through the right leaf of the diaphragm into the right side of the mediastinum where it formed a prominent mass. The inferior vena cava (Fig. 1) was filled with thrombus that extended up to the hepatic veins and down to the iliacs. In the mesentery (Fig. 2) there were many veins filled with thrombi. In the pelvic region around the bladder and around the prostate several veins were also filled with antemortem thrombi. Metastases were found also in the pleura of the left lung, in the spleen, and in the right kidney. The testes were grossly abnormal in weight and appearance, weighing 30 gm. each. The heart (400 gm.) was grossly

normal. There was gross evidence of metastases in the vertebral bone marrow.

Dr. Eduardo Porta: The microscopic examination of the hepatic tumor described grossly showed nodules formed by fairly well differentiated canaliculi and acini and lined by simple columnar epithelium. (Fig. 3.) Some nodules presented necrotic centers and in the viable regions exhibited nuclear dispolarity. (Fig. 4.) This pattern is typical of a cholangioma. The interstitial stroma was moderately abundant and poorly vascularized. These cholangiomas were more scirrous than hepatomas. (Fig. 5.) The stroma at the periphery of a nodule was fibrous, insinuating itself between the liver plates. (Fig. 6.) As one can expect of a tumor formed mainly from ductular cells, no bile formation was found. In some areas the tumor cells arranged themselves in an acinar way. When the latter arrangements are common the diagnosis of hepatocholangioma is made, but in this case acini were not frequent. About twothirds of the cases of cholangiomas exhibit extrahepatic metastasis. Metastasis to the bone however is not frequent. In our case, we have metastases to the vertebral bone marrow, left lung, and pleura, hilar lymph nodes, spleen, kidney, peritoneum, and an extension of the tumor to the right adrenal and to the right leaf of diaphragm. In the vertebral bone marrow the metastases were very sclerotic. The right adrenal was totally destroyed by tumor. (Fig. 7.)

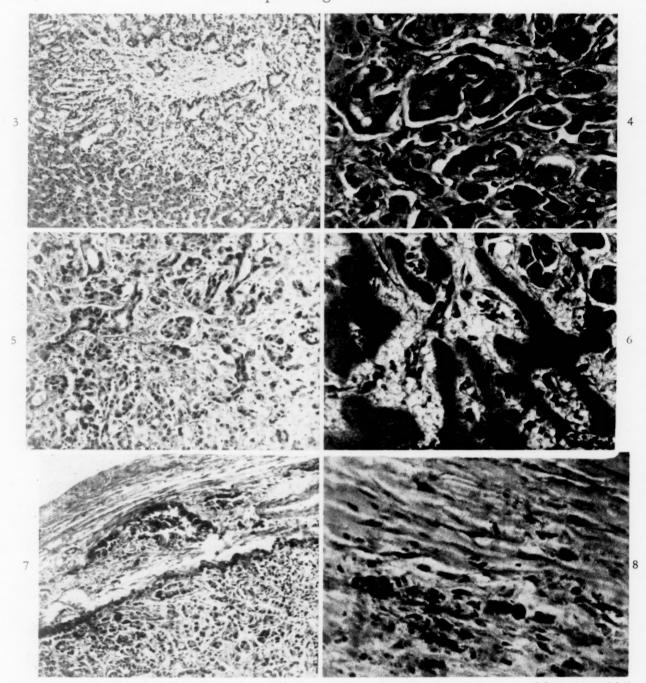


Fig. 3. A low power of section of the liver stained with hematoxylin and eosin to show the pattern of the tumor with its abundant stroma and acinar arrangement of epithelial-lined spaces.

- Fig. 4. A high power of the cholangiole-carcinoma found in the patient's liver to demonstrate nuclear abnormalities and dispolarity. High power photomicrograph of section stained with hematoxylin and eosin.
- Fig. 5. The tumor possesses a very abundant stroma as shown in this medium-power photomicrograph of a hematoxylin and eosin stained-section of the liver.
- Fig. 6. At the edges of the tumor masses, there is stroma penetrating between attenuated cords of atrophic liver cells in the manner of fingers groping outward as shown in this high power photomicrograph of a hematoxylin and eosin stained section.
- Fig. 7. The left adrenal was completely replaced by tumor metastasis. Tumor cells not only replaced the entire cortex as shown here but also invaded the capsule also. Low power photomicrograph of a hematoxylin and eosin-stained section of the adrenal.
- Fig. 8. Section from the inferior vena cava demonstrating the wall of a recanalized space in a thrombotic mass in which several tumor cells can be seen below and to the left in this hematoxylin and eosin-stained section.

In a section from the inferior vena cava, was a recanalized thrombus in which a few tumor cells were present. (Fig. 8.)

The final anatomic diagnoses were: cholangioma (slightly mucin producing) (Surg. Path. #60–1743) involving liver, right adrenal, right leaf of diaphragm and right lower mediastinum; metastases to spleen, kidney, left lung and pleura, peritoneum—vertebral bone marrow and hilar lymph node; tumor thrombosis of inferior vena cava; thrombosis of portal and pelvic veins; right pleural effusion, blood

tinged (2,000 cc); ascites, blood tinged (300 cc); atelectasis of the right lung; pneumonia, upper lobe of left lung; recent infarct of lung, upper lobe of left lung; recent infarcts, both kidneys; edema of legs, bilateral; atrophy of testis, moderate; passive congestion of spleen and pancreas, moderate; atherosclerosis of aorta, slight, and of coronary arteries, moderate; subpleural calcified nodules, left and left hilar calcified lymph node; calcified nodule in spleen; lipoid depletion of the left adrenal.

Stromal Endometriosis Involving the Heart*

HENRY FELSON, M.D., JOHNSON McGuire, M.D. and Philip Wasserman, M.D. Cincinnati, Ohio

This paper describes what we believe to be the first reported instance of involvement of the heart muscle by stromal endometriosis. Progressive cardiac disturbance was responsible for the death of the patient.

The term "endometriosis" indicates ectopic uterine mucosa, characterized by the presence of endometrial glandular and stromal elements. In stromal endometriosis stromal cells prevail and there is no epithelial component.

The clinical course of stromal endometriosis may differ in some respects from that observed in the usual case of endometriosis. In most instances the disorder occurs during the second half of reproductive life or after the menopause. There may be recurrence many years after removal of all apparent lesions, despite resection of the ovaries. Although benign histologically, stromal endometriosis may extend through the uterine wall into the bladder and pelvic wall and may grow around the ureters and constrict them. Distant metastases have been described in some patients; rarely, pulmonary lesions have been noted [1-3]. We have found no report of cardiac involvement. Death has occasionally resulted from the disease, occurring many years after onset, due to the pressure effects of large pelvic lesions.

CASE REPORT

In August 1951, the patient, a previously healthy forty-six year old woman, entered the hospital because of vaginal bleeding. Supravaginal hysterectomy was performed because of an enlarged uterus. The uterus contained fibromyomas. Microscopic examination of uterine sections revealed stromal endometriosis which appeared to be localized to a 6 cm. area of one wall. (Fig. 1.)

In November 1952, physical examination and an electrocardiogram were within normal limits. In May 1954, when the patient was examined by an internist, no cardiac abnormality was noted. In December 1954,

she consulted a gynecologist because of spotty vaginal bleeding. The left side of the cervix was firm, irregular and nodular and a cervical polyp was noted. A biopsy specimen revealed stromal endometriosis in the cervix.

When seen in January 1955, the patient complained of occasional dyspnea on exertion and palpitation during the preceding two months. Examination of the heart revealed a grade 3 systolic murmur in the pulmonic region; P₂ was accentuated. Roentgenographic examination of the heart revealed no abnormality and an electrocardiogram was within normal limits. Because of growth of the cervical lesions and spread to the adjacent fornix, radiation therapy was administered to the ovaries for the purpose of sterilization. By February 13, 1955, the pelvic mass had almost disappeared; only a minor degree of residual induration was felt in the left vaginal wall and the cervix.

On May 10, 1955, there was pain in the back of the throat, jaw, chin and pretracheal region and the patient was hospitalized. Blood pressure was 135/90 mm. Hg; the cardiac findings were unchanged. Roentgenograms of the chest and cardiac fluoroscopy revealed no abnormalities. The pain disappeared within forty-eight hours and she was discharged on June 18, 1955.

On July 6, 1955, the patient was readmitted with historical, physical and electrocardiographic findings (Fig. 2) indicative of pericarditis. She was discharged on August 24, 1955, improved although vague discomfort in the chest persisted. On September 22, 1955, she was again admitted with pericarditis. Pleural effusion in the left side of the chest appeared. (Fig. 3.) The evidence of pericarditis and pleurisy gradually disappeared over a three week period. In December 1955, marked bilateral "shoulder-hand" syndrome developed. Therapy with estrogen (Premarin, 2.5 mg. daily) was instituted because of severe hot flashes. Pelvic examination on this admission revealed no change.

On May 5, 1956, a mass the size of an orange was palpated in the right adnexal region. In addition, dark blue, grape-like clusters of tissue were observed to arise from the cervix and left side of the vagina, and to fill the entire vagina. Therapy with estrogen, which had been administered continuously, was

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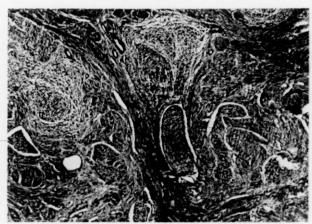


Fig. 1. Surgical specimen obtained in 1951. Endolymphatic stromal endometriosis in myometrium with extension into large lymphatic channels. Original magnification × 60.

immediately discontinued. Fluoroscopy of the heart revealed some enlargement, predominantly right-sided. The relatively clear lung fields and prominent pulsations of the pulmonary artery suggested the existence of pulmonary hypertension. During the next three months a striking reduction in size of the pelvic and vaginal masses was observed and by August, 1956, they had almost disappeared. During 1956 the patient led a very restricted existence, sitting about the house and occasionally taking a walk. She complained of fatigue, weakness, dyspnea and nocturnal sweating.

On November 21, 1956, the patient was admitted to the hospital because of slight right-sided pleural pain and dyspnea. Her temperature was 99.6°F. and her pulse was 104 per minute. The systolic pulmonic murmer was quite loud but P2, formerly accentuated, was inaudible. Physical manifestations of tricuspid insufficiency appeared. There were no signs of pulmonary edema. Her blood pressure was 95/80 mm. Hg. The venous pressure was 14 cm. of water. Fluoroscopic examination revealed absence of pulsation in a localized segment along the left border of the heart. Infiltration, believed to be due to pulmonary infarction, was seen in the right costophrenic sulcus in a roentgenogram of the chest. (Fig. 4.) An electrocardiogram (Fig. 5) revealed marked right axis deviation.

The patient's condition gradually deteriorated. Weakness became more severe. On four occasions there were episodes of syncope following as little effort as changing her position in bed. Slight edema developed but disappeared when mercurial diuretics were administered. During the last few days of life her blood pressure was difficult to obtain. The systolic blood pressure was 80 to 90 mm. Hg, with a very narrow pulse pressure. Death occurred suddenly on December 28, 1956. She was always clear mentally.

At postmortem the important lesions were in

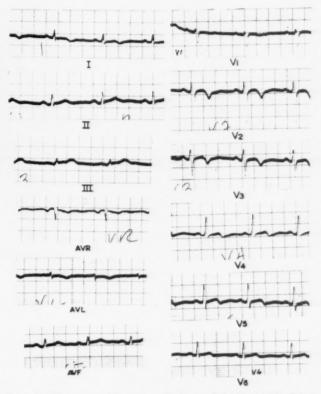


Fig. 2. Electrocardiogram taken on July 9, 1955. The mean QRS axis in the frontal plane is located at approximately plus 60 degrees. There is a small S wave in lead 1 which was not previously present. There is symmetrical T wave inversion in leads 1, aVL, V2, V3, V4 and V5. The T wave changes are compatible with, although not diagnostic of, chronic pericarditis.

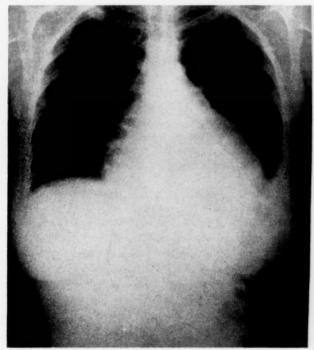


Fig. 3. Roentgenogram taken on September 26, 1955, showing pericardial effusion and pleural effusion.

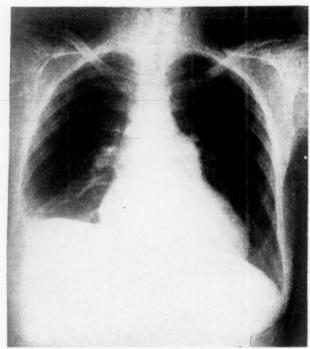


Fig. 4. Roentgenogram obtained on December 21, 1956, showing cor pulmonale and pulmonary infarct, right costophrenic angle. Diminished vascular markings are present in both lung fields.

the heart and in the organs remaining in the pelvis. Other organs, including the lungs, were unaffected.

The heart weighed 560 gm. and the right ventricular wall was clearly hypertrophied. Its external contour was altered by a soft, yellowish tumor mass rising 1.5 cm. above the level of the epicardium on the anterior surface over a 7 cm. area. Yellowish-gray spongy neoplastic tissue extended from the interventricular septum into the right ventricle and expanded to form a sausage-shaped mass measuring 9 by 2 cm. which filled the chamber. The main pulmonary artery was also filled by a freely movable finger-like mass continuous with the ventricular lesion. (Fig. 6.) The tumor also had invaded the wall of the right ventricle to reach the epicardial surface in some places. The left ventricle close to the interventricular septum was invaded to a lesser degree. The lesion varied in texture, being compact and fibrous in some portions and gelatinous or spongy in others. Neoplastic tissue interdigitated between myocardial fibers and appeared to displace, rather than replace, them. The left ventricle was relatively small and somewhat narrowed by obtrusion of the displaced septum.

Microscopic examination revealed two patterns: (1) In the intramural portion the lesion was of compact texture. It was well vascularized and was composed of solid sheets of small, uniform, stromal cells.

The cells were usually elongated and often fusiform or spindle-shaped. The cytoplasm was pale, nuclei relatively small and not heavily stained; mitotic figures were very rarely seen. (2) The polypoid portion contained an outer zone composed of spindle-shaped and round cells. The deeper regions exhibited considerable degeneration and edema characterized by a poorly cellular central area. In many regions the structure of the neoplasm was identical with that of the stromal endometriosis in the sections of the uterus; cleavage was apparent between neoplastic tissue and myocardial fibers whenever the two were in contiguity. In only a few areas was there any indication that the muscle was destroyed by the process. (Fig. 7.)

The only abnormal pelvic structure remaining was

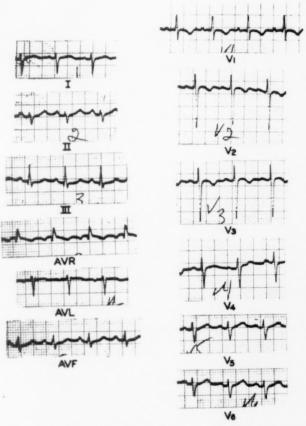


Fig. 5. Electrocardiogram obtained on December 19, 1956. Marked right axis deviation has developed and is characterized by a deep S wave in lead I. The mean QRS axis in the frontal plane is now located at plus 165 degrees. Deep S waves are present in leads V_{δ} and V_{δ} and a relatively tall R wave is present in lead V_{1} . These changes in the QRS complexes indicate the development of right ventricular hypertrophy. There is symmetrical T wave inversion in leads V_{1} through V_{δ} which again might be compatible with chronic pericarditis but which also might be secondary to the development of right ventricular hypertrophy.

the stump of the cervix. This measured 3 by 3 by 4 cm. in diameter. There were accumulations of clot-like material, ranging from pinhead size to about 5 mm. in diameter, at the anterior cervical lip and at the regional vaginal wall. The fallopian tubes and ovaries appeared atrophic but otherwise normal. Microscopic sections of the cervix revealed irregular nests of stromal endometriosis identical with that observed in the previously excised uterus. Neither anaplasia nor increased mitotic activity was seen. The general resemblance to the cardiac lesion was striking.

Death was believed due to occlusion of the major pulmonary artery by the tumor.

COMMENTS

Endolymphatic stromal endometriosis differs from ordinary endometriosis in ways other than the absence of glandular elements. It is well known to be invasive by way of the lymphatic channels and may be associated with extensive pelvic lesions. Distant metastases rarely occur. In this patient, there was an extension to the vagina which is uncommon. In addition, there



Fig. 6. Heart. A polypoid mass extends from the right ventricular wall and appears as a large sausage-shaped lesion protruding into the pulmonary artery.

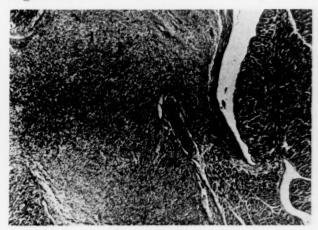


Fig. 7. Stromal endometrioma in the myocardium. Note the sharp line between intact heart muscle and the stromal neoplasm. Original magnification \times 60.

was a unique complication of metastasis to the heart.

The lesions in the cervix and adjacent vagina appeared to be responsive to stimulation by hormones. Their size was reduced when the patient was sterilized by radiation. Later, however, during prolonged therapy with estrogen for severe menopausal symptoms there was a marked enlargement of the cervical and vaginal lesions. These lesions diminished rapidly when administration of estrogens was discontinued. Nevertheless, the cardiac lesion continued to grow.

Since the cervix and uterus often are both affected simultaneously in this disorder, a total hysterectomy should be performed [2,3]; it is possible that dissemination of the tumor could have been prevented in this patient by this procedure. Cases have been reported in which the lesion remained localized for long periods of time despite incomplete removal of the uterus.

Dr. Arthur T. Hertig, Shattuck Professor of Pathological Anatomy, Harvard Medical School, reviewed this case and made the following comment: "This is indeed a unique specimen in our experience. We have seen a good many examples of endolymphatic stromal myosis (stromatous endometriosis) in the myometrium and pelvic tissues. We know some fatal cases exist, but we have never before personally seen any metastasis beyond the pelvis and certainly none in the heart. The tumor certainly appears at home in the muscle. The small infiltrating areas at the margin of the main masses strikingly resemble the original myometrial lesion."

SUMMARY

A case of stromal endometriosis with fatal cardiac involvement is described. Obstruction to blood flow through the right ventricle and the pulmonary artery occurred and caused death.

Acknowledgment: We are indebted to Dr. Arthur Hertig for reviewing the microscopic sections. We are also indebted to Dr. Noble

Fowler and Dr. Edward Gall for their invaluable assistance in the preparation of this manuscript.

REFERENCES

1. PARK, W. W. The nature of stromatous endometriosis.

J. Obst. & Gynaec. Brit. Emp., 56: 759, 1949.

2. Hunter, W. C., Nohlgren, J. E. and Lancefield, S. M. Stromal endometriosis or endometrial sarcoma. Am. J. Obst. & Gynec., 72: 1072, 1956.

3. Stearns, H. C. A study of stromal endometriosis. Am. J. Obst. & Gynec., 75: 663, 1958.

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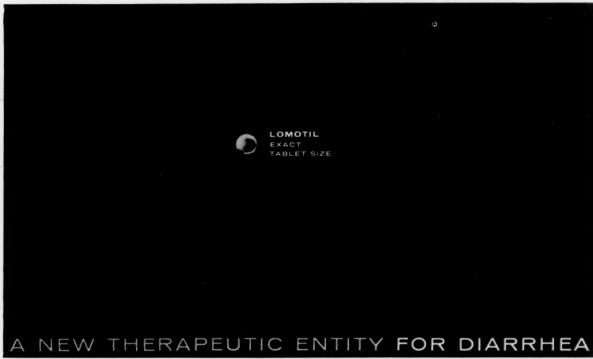
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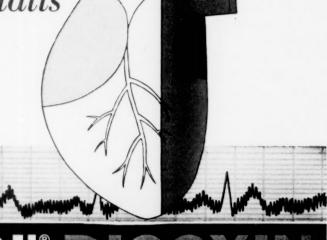
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Lown, B., and Levine, S. A.: Current Concepts in Digitalia Therapy, Boston, Little, Brown & Company, 1954, p. 23, par. 2.

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Cosa-Terramycin provides oxytetracycline (Terramycin®) with glucosamine for enhanced absorption. The dependability of Cosa-Terramycin derives from the broad antimicrobial effectiveness, excellent toleration, and low order of toxicity of oxytetracycline. Pharmacologically, it is characterized by high tissue penetration, low-serum binding, and rapidly attained high urinary concentration.

INDICATIONS: Because oxytetracycline is effective against both gram-positive and gram-negative bacteria, rickettsiae, spirochetes, large viruses, and certain parasites (amebae, pinworms), Cosa-Terramycin is indicated in a great variety of infections due to susceptible organisms, e.g., infections of the respiratory, gastrointestinal, and genitourinary tracts, surgical and soft-tissue infections, ophthalmic and otic infections, and many others.

ADMINISTRATION AND DOSAGE: Adults: 1 Gm. of oxytetracycline daily in four divided doses is usually effective. In *severe* infections, a larger dosage (2-4 Gm. daily) may be indicated. Infants and children: 10-20 mg. of oxytetracycline per lb. of body weight daily. Certain diseases are treated in courses.

For intramuscular therapy: Adults: Terramycin Intramuscular Solution (200-300 mg. daily) should be adequate for most mild and moderately severe infections. In severe infections, 300-500 mg. daily may be necessary. Infants and children, proportionately less.

SIDE EFFECTS AND PRECAUTIONS: Antibiotics may allow overgrowth of nonsusceptible organisms—particularly monilia and resistant staphylococci. If this occurs, discontinue medication and institute indicated supportive therapy and treatment with other appropriate antibiotics. Aluminum hydroxide gel has been shown to decrease antibiotic absorption and is therefore contraindicated. Glossitis and allergic reactions are rare. There are no known contraindications to glucosamine.

SUPPLIED: Cosa-Terramycin Capsules, 250 mg. and 125 mg. Terramycin is also available in: Cosa-Terrabon® Oral Suspension, a palatable preconstituted aqueous suspension containing 125 mg. per 5 cc. teaspoonful, bottles of 2 oz. and 1 pint; Cosa-Terrabon® Pediatric Drops, a palatable preconstituted aqueous suspension containing 5 mg. per drop (100 mg. per cc.), bottle of 10 cc. with calibrated plastic dropper; and Terramycin Intramuscular Solution, conveniently preconstituted, in the new 10 cc. multi-dose vial, 50 mg. per cc., and in 2 cc. prescored glass ampules, containing 100 mg. or 250 mg., packages of 5 and 100. In addition, a variety of other systemic and local dosage forms are available to meet specific therapeutic requirements.

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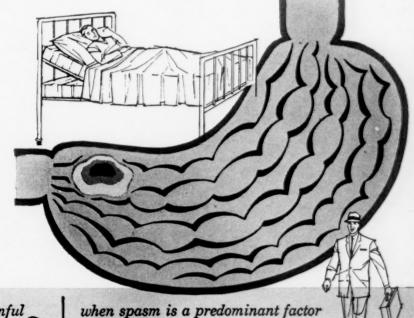
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REFERENCES: (1) Wells, B. B.: Clinical Pathology: Application and Interpretation, ed. 2, Philadelphia, Saunders, 1956, p. 233. (2) Duncan, G. G.: Diseases of Metabolism: Detailed Methods of Diagnosis and Treatment, ed. 4, Philadelphia, Saunders, 1959, p. 795. (3) Williamson, P.: Practical Use of the Office Laboratory and X-ray, Including the Electrocardiograph, St. Louis, Mosby, 1957, p. 41. (4) Mehlman, J. S.; Zitman, I. H., and Platt, S. S.: M. Clin. North America 43:615 (March) 1959.

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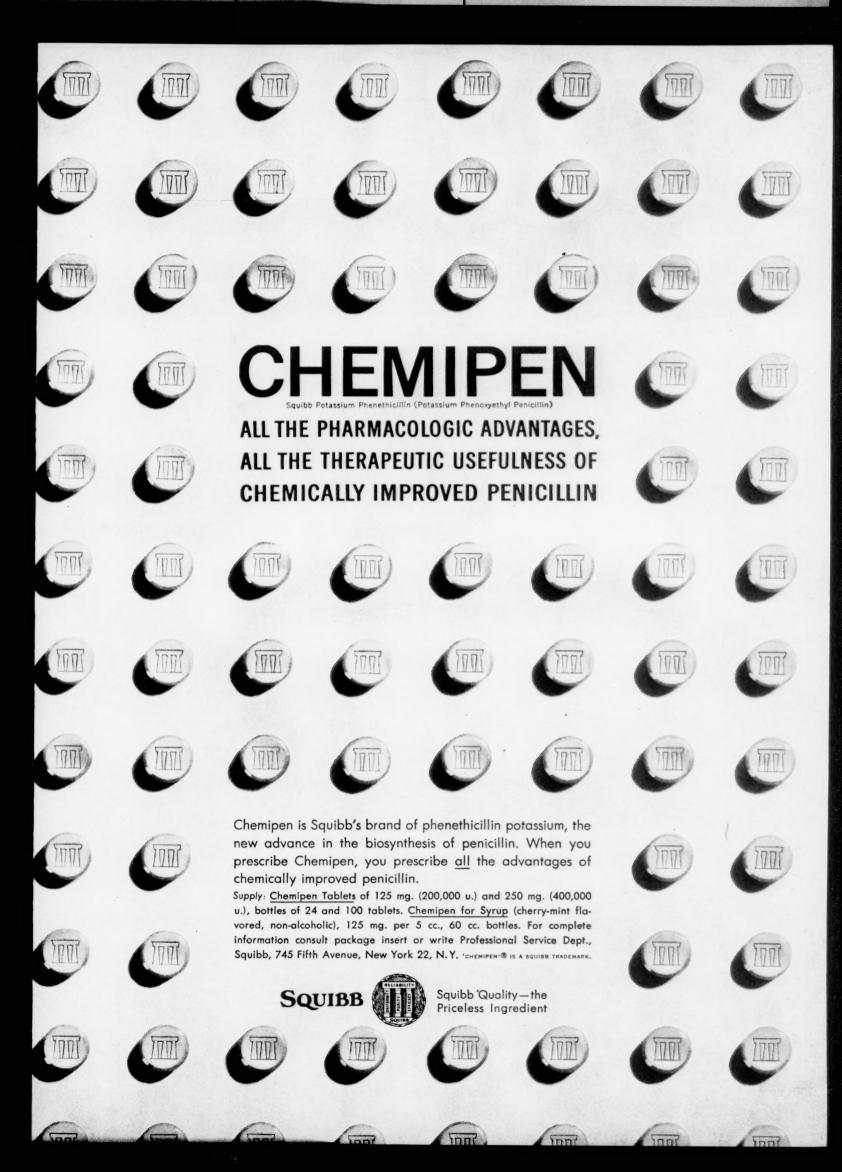






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1. Blumberg, N. et al.: Fed. Proc. 17:351, March 1958. 2. Boyd, L. J. et al.: Am. J. Cardiol. 3:229, Feb. 1959. 3. Brick, H. et al.: J. Social Therapy 4:190, 1958. 4. Bulla, J. D. et al.: Am. Proct. & Digest Treat. 10:1961, Nov. 1959. 5. Ewing, J. A. and Haizlip, T. M.: Am. J. Psychiat. 114:835, March 1958.

6. Friedman, A. P.: Ann, N. Y. Acad. Sc. 67:822, May 9, 1957. 7. Greenberg, L. A. et al.. Ann. N. Y. Acad. Sc. 67:816, May 9, 1957. 8. Holliday, A. R.: Northwest Med. 58:837, June 1959. 9. Hollister, L. E. et al.: Dis. Nerv. System 17:289, Sept. 1956. 10. Loird, D. M. et al.: Dis. Nerv. System 18:346, Sept. 1957.

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11. Lasagna, L.: J. Chron. Dis. 3:122, Feb. 1956. 12. Muhlfelder, W. J. et al.: Dis. Nerv. System 20:587, Dec. 1959. 13. Pollak, M.: Practitioner 184:231, Feb. 1960. 14. Rickels, K. et al.: J.A.M.A. 171:1649, Nov. 21, 1959. 15. Russek, H. I.: Am. J. Cardiol. 3:547, April 1959. 16. Tucker, K. and Wilensky, H.: Am. J. Psychiat. 113:698, Feb. 1957.

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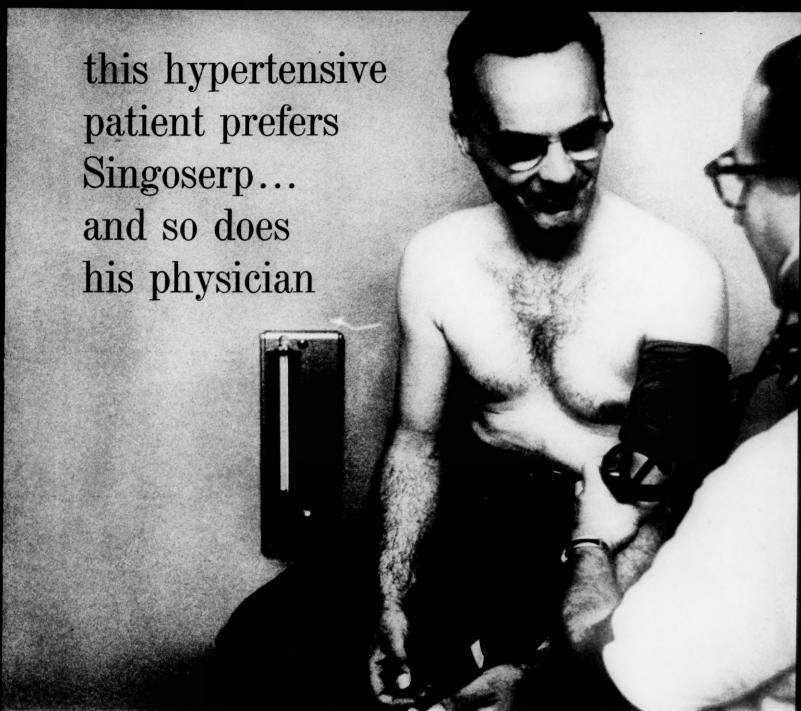


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Vistaril is a rapid-acting calmative with a wide margin of safety. Not a cortical depressant, Vistaril permits the patient to remain calm without dulling mental alertness. It is effective in the symptomatic treatment of a variety of neuroses and other emotional disturbances manifested by anxiety, apprehension or fear — whether occurring alone or complicating a physical illness.

ACTIONS & INDICATIONS: Vistaril, by allaying fear and apprehension, can safely relax your patient and is a valuable aid in the management of agitation and hysteria.

ADVANTAGES: Vistaril produces a calming effect without hypnosis. Vistaril provides direct and secondary muscle relaxation. Vistaril apparently is nonaddicting—discontinuance after months of treatment has not produced withdrawal symptoms. Vistaril has a remarkable record of safety when used in recommended dosage. Unlike the phenothiazines, parkinsonism and blood or liver toxicities have not been reported with Vistaril. Unlike the rauwolfia derivatives, Vistaril acts rapidly, does not increase gastric secretions, and there have been no reports of nasal congestion, drug-induced depression, or sinusitis associated with its use. Unlike the meprobamates, there have been no reports of addiction, incoordination, ataxia, abdominal discomfort, anorexia, nausea, vomiting, diarrhea, allergic dermatitis, or anaphylactic reactions. VISTARIL PARENTERAL SOLUTION permits rapid action and may be given via I.M. or I.V. routes.

CONTRAINDICATIONS: There are no known contraindications to Vistaril.

SIDE EFFECTS AND PRECAUTIONS: Drowsiness may occur in some patients; if so, it is usually transitory, disappearing upon reduction of dosage or within a few days of continued therapy. Dryness of mouth may be encountered at higher dosages. The potentiating action of hydroxyzine must be taken into consideration when it is used in conjunction with C.N.S. depressants. Do not exceed 1 cc. per minute I.V. Do not give over 100 mg, per dose I.V. Parenteral therapy is for 24-48 hours, unless changed by judgment of physician.

ADMINISTRATION AND DOSAGE: Vistaril dosage varies with the state and response of each patient, rather than on a weight basis. Dosage should be individualized by the physician for optimum results. For adult psychiatric and emotional emergencies, including acute alcoholism, the following dosage is suggested: I.M.—25-100 mg. Stat., and q. 4-6 h., p.r.n.; I.V.—50 mg. Stat., maintain with 25-50 mg. I.V. q. 4-6 h., p.r.n.

HOW SUPPLIED: Vistaril Parenteral Solution—10 cc. vials and 2 cc. Steraject® Cartridges, 25 mg. per cc.; 2 cc. ampules, 50 mg. per cc. Vistaril Capsules (as the pamoate)—25, 50, and 100 mg. Oral Suspension (as the pamoate)—25 mg. per 5 cc. teaspoonful.

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References: (1) Bauer, A. W.; Perry, D. M., & Kirby, W. M. M.: J.A.M.A. 173:475, 1960. (2) Goslings, W. R. O., & Büchli, K.: Arch. Int. Med. 102:691, 1958. (3) Goodier, T. E. W., & Parry, W. R.: Lancet 1:356, 1959. (4) Fisher, M. W.: Arch. Int. Med. 105:413, 1960. (5) Petersdorf, R. G., et al.: Arch. Int. Med. 105:398, 1960. (6) Glas, W. W., in Symposium on Antibacterial Therapy, Michigan & Wayne County Acad. Gen. Pract., Detroit, September 12, 1959, p. 7. (7) Modarress, Y.; Ryan, R. J., & Francis, Sr. C. E: J. M. Soc. New Jersey 57:168, 1960. (8) Rebhan, A. W., & Edwards, H. E.: Canad. M. A. J. 82:513, 1960.

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1957	96%
1958	95%
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*Adapted from Bauer, Perry, & Kirby1

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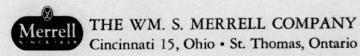
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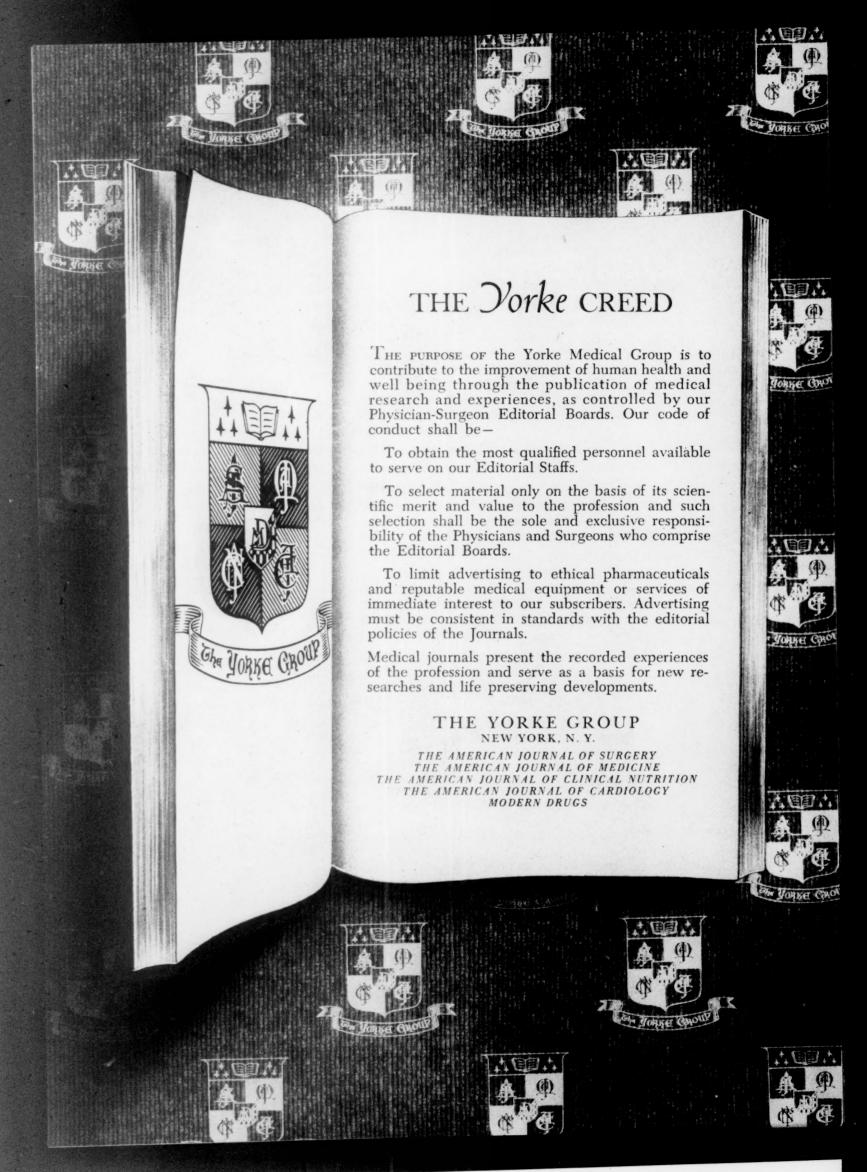
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References: 1. Hollander, W., and Chobanian, A. V.: Boston M. Quart. 10:37 (June) 1959. 2. Oaks, W., and Lisan, P.: Fed. Proc. 18:428 (Mar.) 1959. 3. Oaks, W. W., et al.: A. M. A. Arch. Int. Med. 104:527 (Oct.) 1959. 4. Lisan, P.: Proceedings, Conference on MER/29, Progr. Cardiovasc. Dis. 2:(Suppl.)618 (May) 1960. 5. Oaks, W. W.: 1bid., p. 612. 6. Hollander, W., et al.: 1bid., p. 637. 7. Halperin, M. H.: 1bid., p. 631. 8. Toro, J.: 1bid., p. 544. 9. Morrison, L. M.: J.A.M.A. 173:884 (June 25) 1960.





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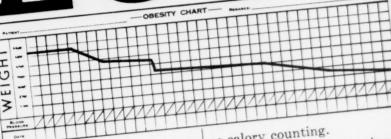
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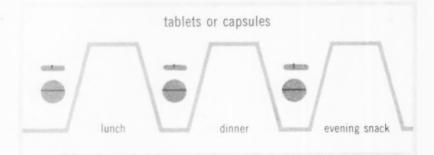
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1. Lehrer, H. W., et al.: Northwest Med. 75:1249, 1955.

2. Smith, Richard T.: New York Med. 8:16, 1952.

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Bibliography (13 clinical studies, 858 patients): 1. Alexander, L. (35 patients): Chemotherapy of depression - Use of meprobamate combined with benactyzine (2-diethylaminoethyl benzilate) hydrochloride. J.A.M.A. 166:1019, March 1, 1958. 2. Bateman, J. C. and Carlton, H. N. (50 patients): Meprobamate and benactyzine hydrochloride (Deprol) as adjunctive therapy for patients with advanced cancer. Antibiotic Med. & Clin. Therapy 6:648; Nov. 1959. 3. Beerman, H. M. (44 patients): The treatment of depression with meprobamate and benactyzine hydrochloride. Western Med. 1:10, March 1960. 4. Bell, J. L., Tauber, H., Santy, A. and Pulito, F. (77 patients): Treatment of depressive states in office practice. Dis. Nerv. System 20.263, June 1959. **5.** Breitner, C. (31 patients): On mental depressions. Dis. Nerv. System 20:142, (Section Two), May 1959. **6.** Gordon, P. E. (50 patients): Deprol in the treatment of depression. Dis. Nerv. System 21:215, April 1960. **7.** Landman, M. E. (50 patients): Clinical trial of a new antidepressive agent. J. M. Soc. New Jersey. In press, 1960. 8. McClure, C. W., Papas, P. N., Speare, G. S., Palmer, E., Slattery, J. J., Konefal, S. H., Henken, B. S., Wood, C. A. and Ceresia, G. B. (128 patients): Treatment of depression - New technics and therapy. Am. Pract. & Digest Treat. 10:1525, Sept. 1959. 9. Pennington, V. M. (135 patients): Meprobamate benactyzine (Deprol) in the treatment of chronic brain syndrome, schizophrenia and senility. J. Am. Geriatrics Soc. 7:656, Aug. 1959. 10. Rickels, K. and Ewing, J. H. (35 patients): Deprol in depressive conditions. Dis. Nerv. System 20:364, (Section One), Aug. 1959. 11. Ruchwarger, A. (87 patients): Use of Deprol (meprobamate combined with benactyzine hydrochloride) in the office treatment of depression. M. Ann. District of Columbia 28:438, Aug. 1959. 12. Settel, E. (52 patients): Treatment of depression in the elderly with a meprobamate-benactyzine hydrochloride combination. Antibiotic Med. & Clin. Therapy 7:28, Jan. 1960. 13. Splitter, S. R. (84 patients): Treatment of the anxious patient in general practice. J. Clin. & Exper. Psychopath. In press, April-June 1960.

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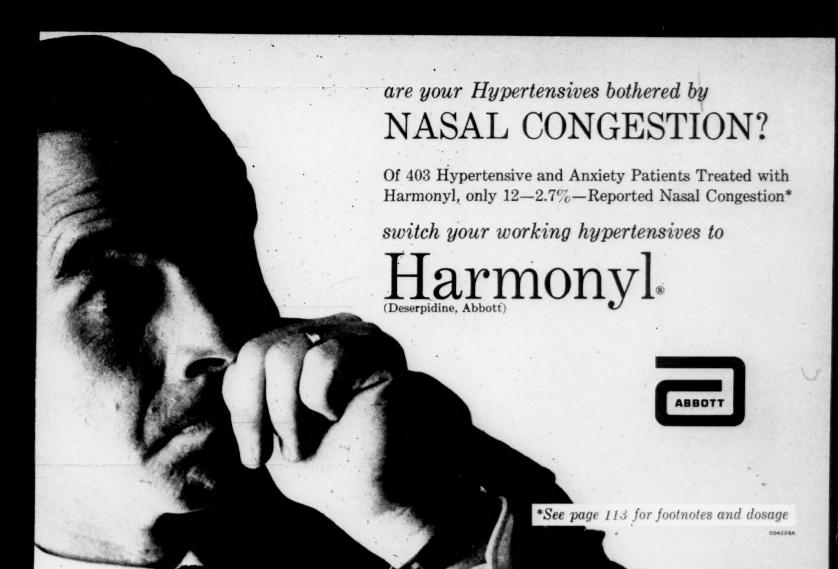
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"Slow clearers" gradually accumulate an excess of fat in the blood stream over a period of years as each meal adds an additional burden to an already fat-laden serum. As shown in figure #2, the blood literally becomes saturated with large fat particles, presenting a dual hazard to the atherosclerotic patient: the long-term danger of deposition of these fats on the vessel walls,⁴ and the more immediate risk of high blood fat levels after a particularly heavy meal possibly precipitating acute coronary embarrassment.⁵

In figure #3, the test tube at the left contains lipemic serum, while the one at the right contains clear, or normal serum. If serum examined after a 12-hour fasting period presents a milky appearance, this is a strong indication that the patient clears fat slowly and is a candidate for antilipemic therapy in an effort to check a potentially serious situation.

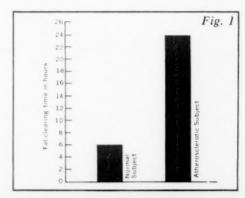
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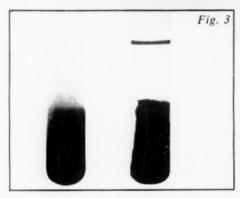
References: 1. Anfinsen, C. B.: Symposium on Atherosclerosis, National Academy of Sciences, National Research Council Publication 338, 1955, p. 218. 2. Berkowitz, D.; Likoff, W., and Spitzer, J. J.: Clin. Res. 7:225 (Apr.) 1959. 3. Stutman, L. J., and George, M.: Clin. Res. 7:225 (Apr.) 1959. 4. Wilkinson, C. F., Jr.: Annals of Int. Med. 45:674 (Oct.) 1956. 5. Kuo, P. T., and Joyner, C. R., Jr.: J.A.M.A. 163:727 (March 2) 1957. 6. Fuller, H. L.: Angiology 9:311 (Oct.) 1958. 7. Shaftel, H. E., and Selman, D.: Angiology 10:131 (June) 1959. 8. Fuller, H. L.: Circulation 20:699 (Oct.) 1959.

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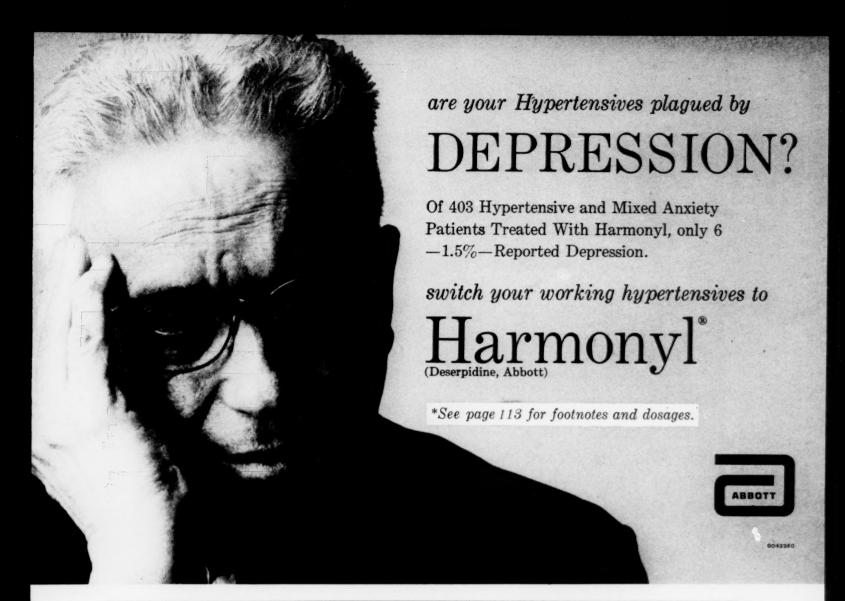
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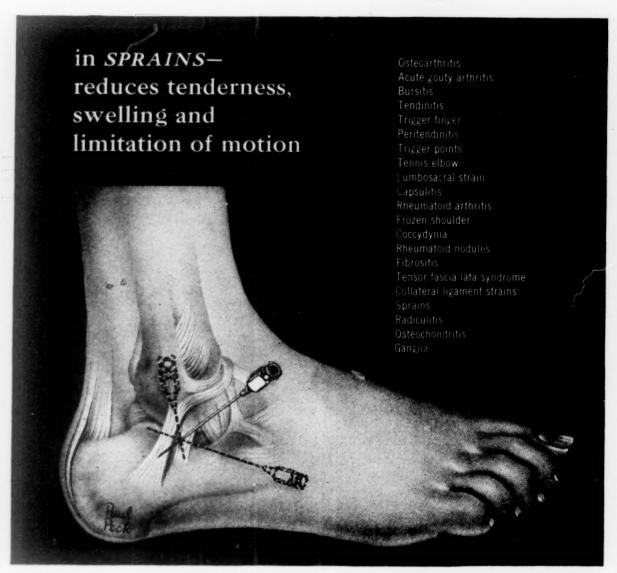
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- Billow, B.W. et al., The Use of a New Rauwolfia Derivative, Description, in Mild Functional Disturbances and Office Psychiatry, N.Y. J. Med., 59:1789, May, 1959.
 Winsor, T., Comparative Effects of Various Rauwolfia Alkaloids in Hypertension, Diseases of the Chest, 35:415, April, 1959.
- April, 1959.
 Rawls, W.B. and Evans, W.L. Jr., Clinical Experiences with Deserpidine in the Management of Hypertension and Anxiety Neurosis, N.Y. J. Med., 59:1774, May, 1959.
 Frohman, I.P., Tranquilizers in General Practice and Clinical Evaluation of Deserpidine, An Alkaloid of Rauwolfia Canescens, Med. Ann. District of Columbia, 27:641, December, 1958.

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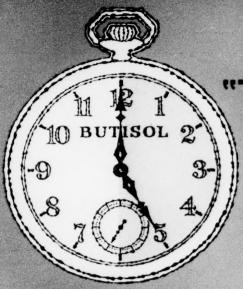
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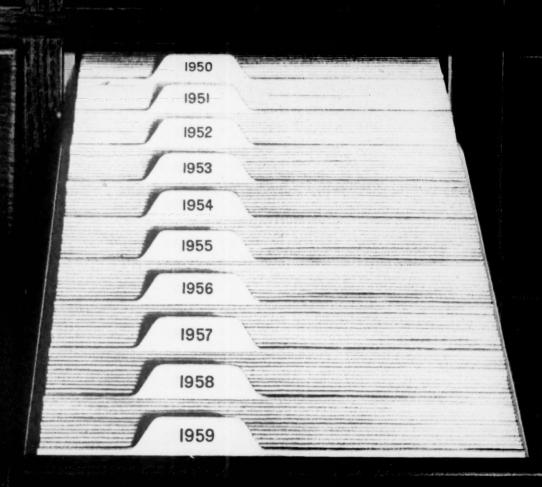
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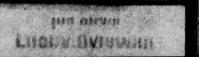


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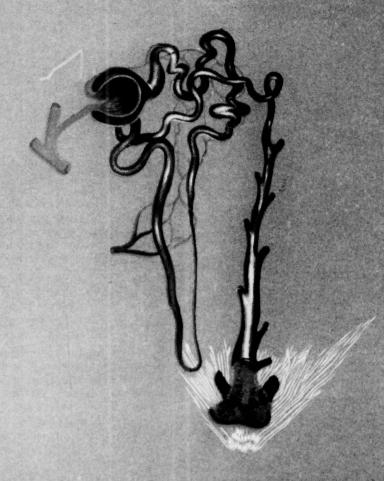
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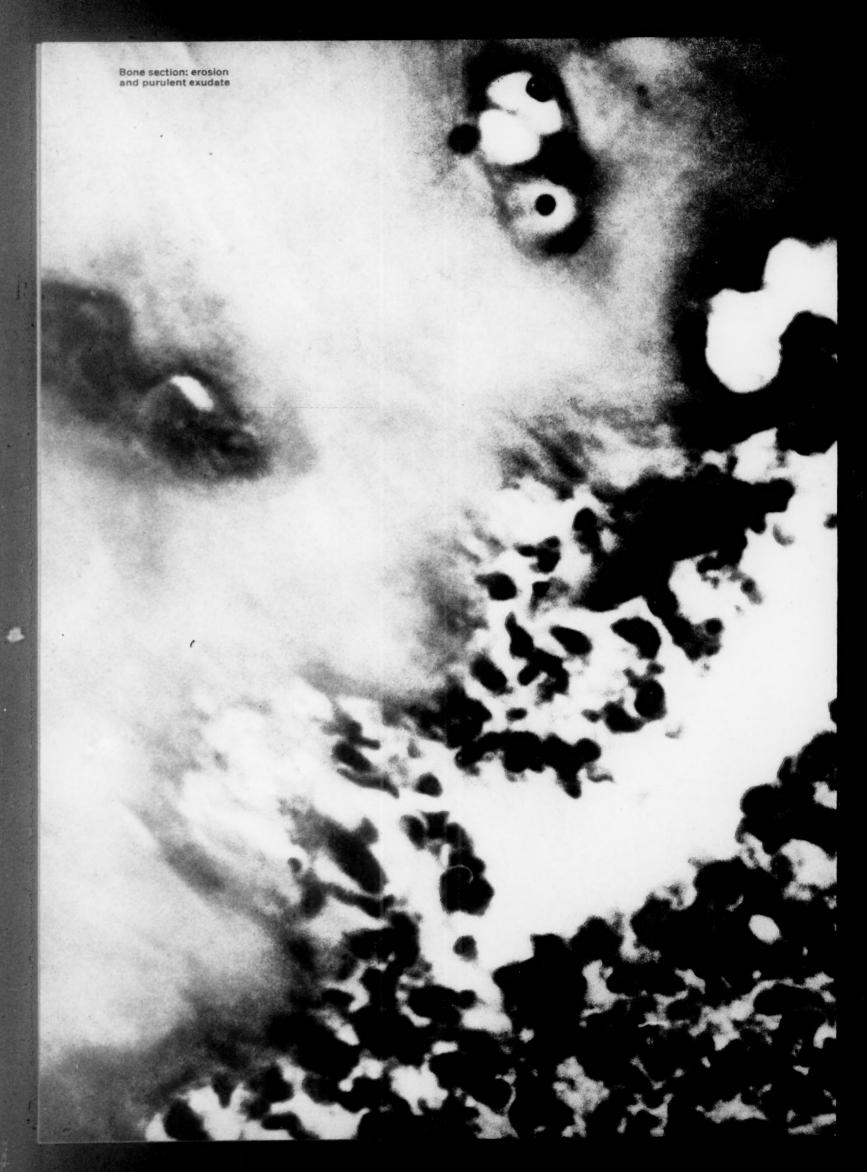
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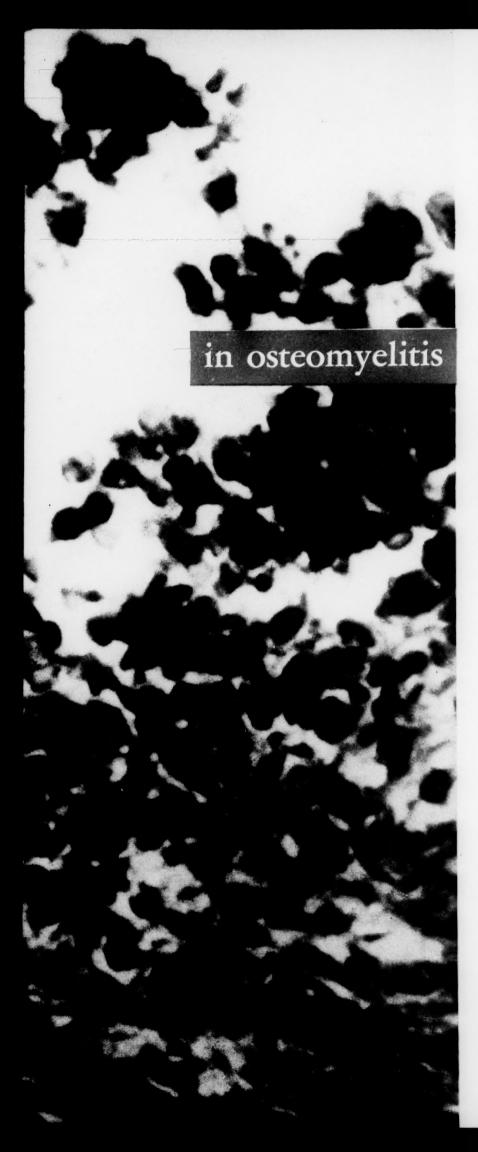
REFERENCES: 1. Schreiner, G. E., A.M.A. Arch. Int. M. 102:32, 1958. 2. Rocha, H., et al.: Yale J. Biol. & Med. 32:120, 1959. 3. Freedman, L. R.: Yale J. Biol. & Med. 32:272, 1960. 4. Freedman, L. R., and Beeson, P. B.: Yale J. Biol. & Med. 30:406, 1958. 5. Rocha, H., et al.: Yale J. Biol. & Med. 30:341, 1958. 6. Paul, M. F., et al.: Am. J. Physiol. 197:580, 1959.



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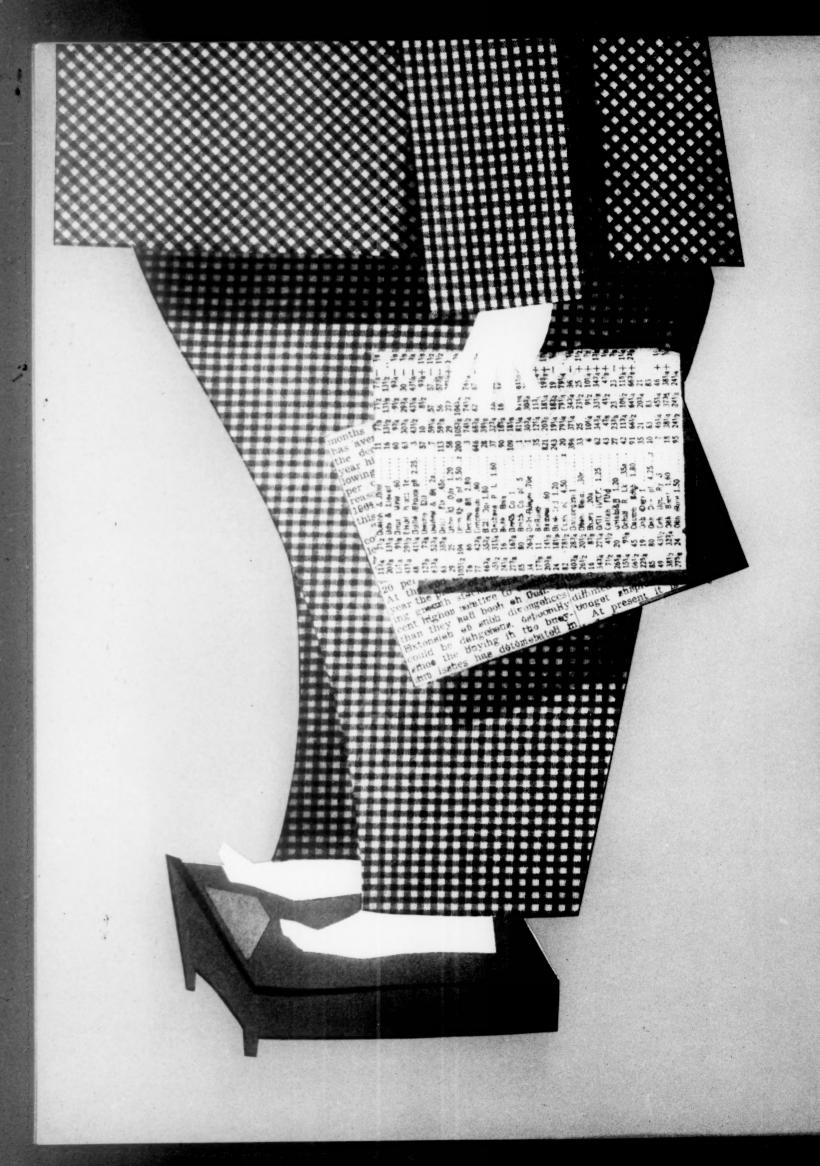
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(3) Roberts, H. J.: Effective Long-Term Weight Reduction—Experiences With Metrecal, to be published.



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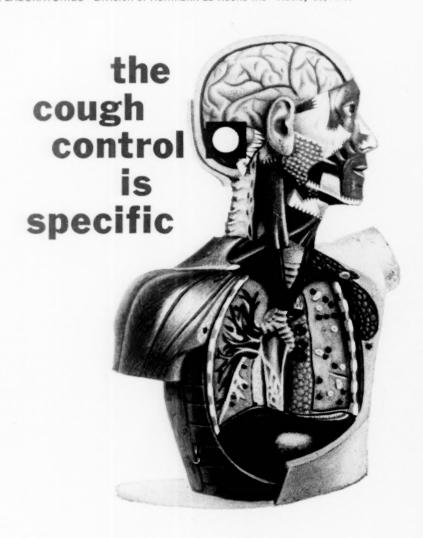
REFERENCES: 1. H. A. Bickerman in W. Modell, Ed., Drugs of Choice 1958-1959, St. Louis, The C. V. Mosby Company, p. 557. 2. L. J. Cass and W. S. Frederik, New England J. Med., 249:132, 1953. 3. L. J. Cass, W. S. Frederik and J. B. Andosca, Am. J. M. Sc., 227:291, 1954. 4. H. Isbell and H. F. Fraser, J. Pharmacol. & Exper. Therap., 107:524, 1953. 5. W. M. Benson, P. L. Stefko and L. O. Randall, J. Pharmacol. & Exper. Therap., 109:189, 1953. 6. New and Nonofficial Drugs 1959, Philadelphia, J. B. Lippincott Company, 1959, p. 326. 7. N. Ralph, Am. J. M. Sc., 227:297, 1954. 8. H. A. Bickerman, E. German, B. M. Cohen and S. E. Itkin, Am. J. M. Sc., 234:191, 1957.

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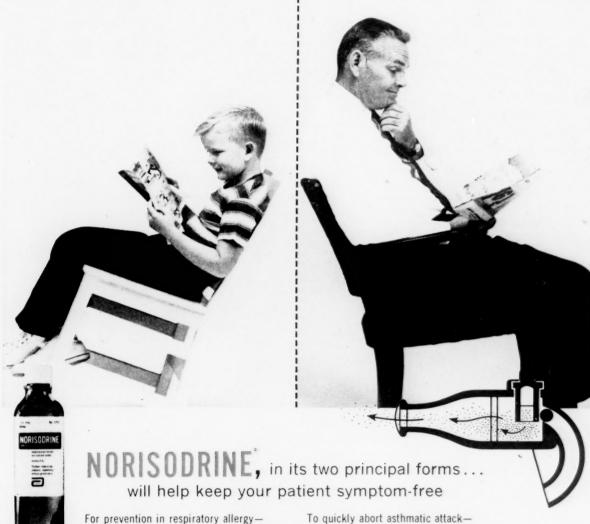
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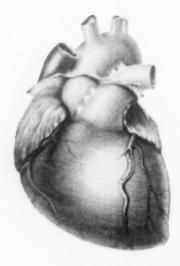
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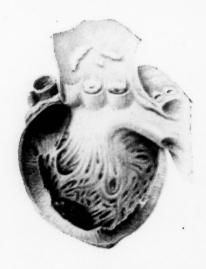
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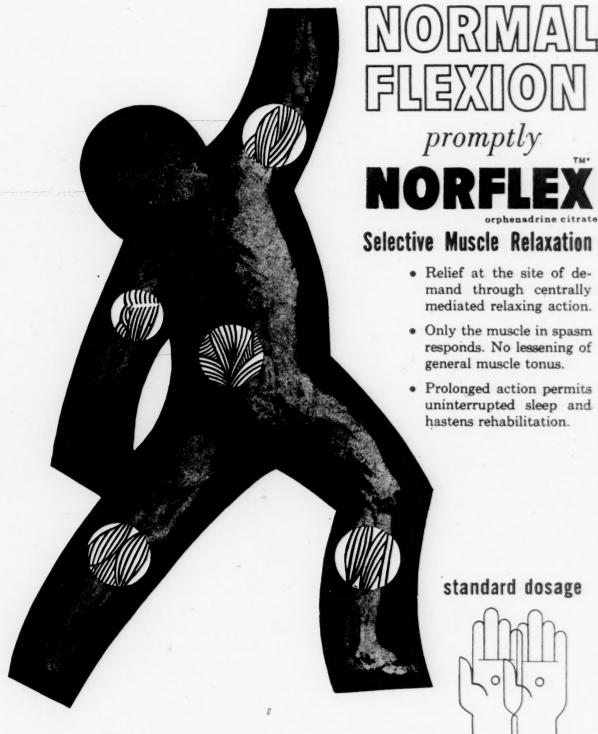
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1. Ellis, L. B. et al.: Circulation 17:945, May 1958. 2. Friedlander, H. S.: Am. J. Cardiol. 1:395, Mar. 1958. 3. Riseman. J. E.F.: New England J. Med. 261:1017, Nov. 12, 1959. 4. Russek, H. I. et al.: Circulation 12:169, Aug. 1955. 5. Russek, H. L.: Am. J. Cardiol. 3:547, April 1959. 6. Tortora, A. R.: Delaware M. J. 30:298, Oct. 1958. 7. Waldman, S. and Pelner, L.: Am. Pract. & Digest Treat. 8:1075, July 1957.

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